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2792 ① biophysical chemistry
② Carbohydrates ③ lipids ④ —
Proteins ⑤ enzymes ⑥ minerals
⑦ foods ⑧ digestion ⑨ absorption ⑩ metabolism ⑪
Calorimetry ⑫ tissues ⑬ Blood
⑭ antibiotics ⑮ urine ⑯ endocrine glands ⑰ vitamins
⑱ biochemistry

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Essentials of
PHYSIOLOGICAL
CHEMISTRY

Essentials of Physiological Chemistry
Fourth Edition

Laboratory Experiments in Physiological Chemistry. *First Edition, Revised Printing*

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Essentials of PHYSIOLOGICAL CHEMISTRY

Fourth Edition

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Preface

In writing this book it has been my purpose to present the more important facts of biochemistry, as related to the animal body, in a form which will be understandable to a student with a limited preparation in chemistry and biology. However, this book presupposes a background of at least a brief course in organic chemistry. It is an outgrowth of courses given to undergraduate students in biochemistry, home economics, premedicine, chemistry, bacteriology, and agriculture.

It is my opinion that a thorough knowledge of the chemistry of biological compounds is a prerequisite to an understanding of biochemistry. For this reason more emphasis is placed on the chemistry of the carbohydrates, lipids, and proteins than is found in many of the elementary books in this field.

Since laboratory work is usually an important feature of a course in biochemistry, I have felt justified in presenting considerable material with laboratory work in view. Many of the questions which students ask in the laboratory are answered in the textbook. In this way, I believe, lecture and laboratory work are more closely tied together.

It has been my experience as a teacher that the undergraduate student makes very little use of references for substantiating statements or for the purpose of stimulating outside reading. For this reason only a few of the more important general references are included at the end of each chapter. If the student refers to these books, he will find in most of them rather complete bibliographies on the subjects concerned.

Many of the subjects considered in this book are controversial. I have attempted, however, to avoid controversy as much as possible, since it seems unwise to confuse the student with arguments pro and con. I have thought it best to leave these for the more comprehensive books used in advanced courses on biochemistry.

Since the publication of the last edition of this book in 1947, many advances have been made in biochemistry. In the present edition I have attempted to bring the subject matter up to date. Many sections have been rewritten, and some have been enlarged for clarity.

Among the more important changes is the addition of a section on isotopes in the chapter on biophysical chemistry. In the chapter on

carbohydrates, Haworth formulas have been included, the section
photosynthesis has been rewritten, and more material is given on t
structure of starch and cellulose. In the chapter on lipids, antioxida
are discussed in greater detail. The chapters on enzymes and carb
hydrate metabolism have been largely rewritten. In the chapter
blood, the sections on bile pigments and respiration have been rewri
ten. The subjects of chemotherapy and adrenal hormones have bee
brought up-to-date. In the chapter on vitamins, more emphasis h
been placed on their physiological action. Recent work on folic aci
vitamin B_{12}, the animal protein factor, and antivitamins is presente

I thank all those who answered my questionnaire regarding sugge
tions for improving this new edition. Many of the suggestions have bee
included in the revision. I wish to acknowledge the assistance of m
daughter Dr. Mary Anderson Hilton of Johns Hopkins University, wh
prepared the chapter on chemotherapy and antibiotics. I want to ex
press appreciation especially to my colleague Dr. Gordon H. Pritha
for his many suggestions. I also wish to thank Dr. R. Adams Dutche
Dr. N. B. Guerrant, Dr. C. O. Jensen, Dr. M. W. Lisse, Mrs. Sarah
McGrew, and Dr. H. O. Triebold for their advice and suggestion
Finally, I wish to acknowledge the assistance of my wife and m
daughter Barbara Anderson Schick in the preparation of the manu
script.

ARTHUR K. ANDERSON

State College, Pennsylvania
April, 1953

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1

Introduction

Every student of organic chemistry is familiar with Wöhler's epoch-making discovery in 1828 of the synthesis of urea from ammonium cyanate. The significance of this discovery was not that a difficult organic synthesis had been performed but rather that it refuted important conceptions regarding the nature of organic compounds. Before Wöhler's discovery organic chemistry concerned itself with the compounds found in living matter, that is, in animals and plants, and it was thought that these compounds bore little relationship to what we now know as inorganic compounds. In fact, it was felt that the compounds found in animals and plants owed their origin to some mysterious vital force which was beyond the scope of human intelligence. Urea was a typical organic compound, according to these early conceptions, since it was the main nitrogenous constituent of human urine, whereas ammonium cyanate was classed as an inorganic compound. The synthesis of urea by simply boiling a solution of ammonium cyanate showed that organic compounds could be made in the laboratory and that a vital force was unnecessary.

An important generalization that may be made concerning the compounds found in animals and plants is that most of them contain the element carbon. Since Wöhler's synthesis of urea hundreds of thousands of carbon compounds have been synthesized in the laboratory, and most of them are in no way associated with living things. Today **organic chemistry** has been expanded to include all carbon compounds and may be defined as the chemistry of carbon and its compounds. The study of the carbon compounds found in animals and plants has become a minor part of organic chemistry.

The branch of chemistry which we are about to consider is called **biochemistry** and may be defined as the chemistry of living matter. Thus in many ways it resembles the old conception of organic chemistry. Since living matter may be divided into two types, namely animal and plant, it is now customary to subdivide biochemistry into animal and plant biochemistry. Animal biochemistry, which is an outgrowth of animal physiology, is usually called **physiological chem-**

istry, whereas plant biochemistry is often referred to as **phytochemistry.**

At one time it was thought that plants and animals were distinctly different in the types of chemical reactions taking place in their tissues. Those going on in plants were considered to be mainly synthetic; those in animals, mainly decompositional. In other words, plants were chiefly concerned with building up complex compounds from the simple raw materials obtained from the soil and air; animals, with the transformation of complex foods into materials for the growth and repair of body tissue and the production of heat and energy. We now know that this difference is mainly quantitative, since, under conditions unfavorable for synthesis, plants oxidize their reserve food supplies, just as animals oxidize their foods. Also animals are constantly synthesizing complex compounds from the simple molecules resulting from the decomposition of foods in the body. Thus, there is no fundamental difference between the chemical processes going on in animals and plants which would necessitate a subdivision of biochemistry.

Both animals and plants are made up of cells, and every living cell is filled with a jellylike, viscous substance called **protoplasm,** which is fundamentally the same whether it is found in animal or plant cells. Protoplasm is the basic substance concerned with life. Biochemistry then becomes the study of the composition of protoplasm and the chemical changes which take place in it.

It is difficult to compose a concise definition of living matter which will distinguish it from lifeless material. Perhaps such distinction can best be made by mentioning certain properties which are common to most living matter but are lacking in lifeless material. First, living things have the power within themselves to **move.** This ability is very evident in animals, which may move from place to place. It is less evident in plants, but a study of their habits by means of high-speed motion pictures has revealed startling movement in plants also. The opening of the flowers of the four-o'clock each afternoon is a good example of motion in a plant. Second, living things **grow;** they increase in size, not as a stone in the bottom of a creek may enlarge by deposition of material upon it, but from within, by increase in the size or the number of cells. Third, living matter is **irritable;** in other words, it responds to stimuli. A living animal moves if pricked with a pin; a dead ones does not. The sensitive plant closes its leaves in response to a touch, and plants in the dark grow toward light. Fourth, living matter has the power of **reproduction.** Living matter which is at present on the earth either must have existed always or must be a product of reproduction from some pre-existing organism. We know that the second alternative is the only reasonable explanation. Fifth, living matter is constantly undergoing chemical changes known as **metabolism.** All the properties of living matter which have been mentioned are the results of metabolism. Growth is the result of the absorption of food and its conversion into new tissue. Motion requires energy, which comes from the oxidation of foods. The oxidation of foods, which involves the utilization of oxygen and the liberation of carbon dioxide, is a prominent phase of metabolism known as *respiration*. Even apparently lifeless tissues like those in seeds, vegetables, and fruits have perceptible respiratory exchanges. An important feature of the metabolism going on in protoplasm is that it is regulated. Chemical changes do not take place in a haphazard manner; they are of a nature suited to the needs of the particular process occurring in a given mass of protoplasm. Of the five properties of living matter just mentioned, metabolism and reproduction are perhaps the most universally accepted criteria of life. We may define living matter as something which has the power of motion, growth, irritability, reproduction, and metabolism. No lifeless thing has all these properties.

Because of the remarkable properties which protoplasm exhibits, some very rare elements might be expected in its make-up. As a matter of fact, protoplasm is composed of the commonest elements found on the earth. Perhaps the most striking thing about the composition of protoplasm is its high water content. The amount of water present varies from 70 to 90 per cent. In general, we may say that the more reactive protoplasm is, the higher is its water content. Inorganic elements in the form of common salts are found in relatively small amounts. About 1 per cent of protoplasm is ash, which is composed of sodium, potassium, calcium, magnesium, iron, phosphates, carbonates, sulfates, and chlorides in comparatively large amounts together with traces of such elements as copper, manganese, zinc, silicon, tin, and iodine.

From 10 to 25 per cent of protoplasm is made up of organic matter. This material may be divided into four main classes of compounds, namely, the carbohydrates, the lipids, proteins, and a miscellaneous group of compounds commonly called extractives.

REVIEW QUESTIONS

1. What achievement of Wöhler's in 1828 changed the conception of the nature of organic chemistry?
2. Define organic chemistry, biochemistry, physiological chemistry, and phytochemistry.
3. Name and discuss the properties of living matter.
4. What are the important constituents of protoplasm?

2

Biophysical Chemistry

In order to discuss intelligently the subjects which are to follow, certain fundamental facts belonging to the field of physical chemistry should be considered. Physics and chemistry are sciences which in many ways are closely related. Physical chemistry might be defined as the science that covers the field where physics and chemistry overlap. There are many places where physics and biochemistry overlap, and a new science has arisen called biophysical chemistry which covers this field. Consider this example: food is oxidized in the body and heat is produced. The oxidation of food is a chemical process, whereas the measurement of the heat produced is a physical problem. Only a few of the more important aspects of biophysical chemistry will be discussed in this chapter.

Properties of Water of Biological Importance. Protoplasm, as was pointed out in Chapter 1, contains from 70 to 90 per cent of water. Many of the properties of water are extremely important in biology. One of water's most biologically significant properties is its high **specific heat.** By specific heat is meant the number of calories needed to raise the temperature of 1 gram of a substance 1°C. More than ten times as much heat is required to raise the temperature of a given weight of water 1°C. as is required to raise the temperature of an equal weight of copper the same amount. In fact, it takes more heat to raise the temperature of a given weight of water 1°C. than is needed for almost any other known substance. Likewise, water gives off more heat when it cools than any other substance.

Water also has a high **heat of vaporization.** By heat of vaporization is meant the heat required to change 1 gram of a liquid into a gas at the same temperature. More than two and one-half times as much heat is required to convert 1 gram of water to a gas as to change 1 gram of ethyl alcohol to a gas. It requires 539 calories to convert 1 gram of water at 100°C. to a gas. When we perspire and water evaporates from the surface of our bodies, there is a cooling effect. These properties of water are undoubtedly of extreme importance to an animal in the functioning of its heat-regulatory mechanism. Water is the best substance we could have in our bodies to aid us in maintaining a constant temperature. It is a remarkable fact that body temperature remains fairly constant in health, and the fact that our bodies have such a high water content is responsible to a considerable degree.

Another property of water which is of great advantage to a living organism is its ability to dissolve substances. No other known liquid is such a **universal solvent** as water. Since all the substances which comprise protoplasm are more or less soluble in water, water makes an ideal medium for carrying nutrients to and waste products from the cells. Protoplasm may be regarded as a combination of a true and a colloidal solution of its various constituents in water.

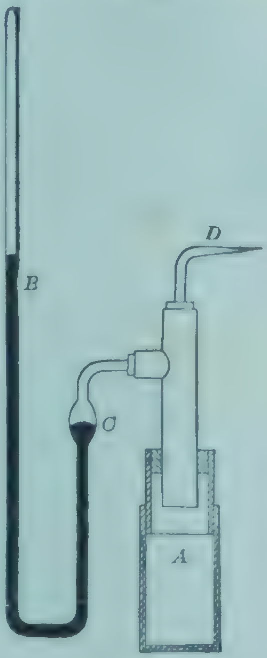


FIG. 1. Apparatus for measurement of osmotic pressure. The porous cup, *A*, with the $Cu_2Fe(CN)_6$ membrane deposited in its walls, contains the solution to be tested. *B* is a mercury manometer. *D* is a glass tube which is sealed when the level of the mercury at *C* is the same as the level of the solution in *A*. The cup *A* is placed in distilled water. From *Introduction to Physiological Chemistry* by Bodansky, John Wiley & Sons, by permission.

Properties of Solutions. If it is true that protoplasm is a solution of its components in water, a knowledge of some of the properties of solutions should be helpful in understanding what is going on in protoplasm.

Diffusion. If a concentrated solution of copper sulfate is placed in a test tube and a layer of water carefully added, it will be noted that in time the copper sulfate will rise until finally the entire liquid has a uniform blue color. The process by which molecules in solution distribute themselves uniformly throughout a solution is called **diffusion.** The rate of diffusion is influenced by such factors as the temperature and the size of particles in the solution. The smaller the particles and the higher the temperature the more rapidly diffusion will take place.

Osmosis and Osmotic Pressure. In many respects a compound in solution conducts itself as though it were a gas. It will be recalled that in elementary chemistry much stress is laid on the gas laws. One of these, known as **Avogadro's law,** states that there is the same number of molecules in equal volumes of all gases at the same temperature and pressure. It will also be remembered that 1 gram molecule of any gas at 0°C. and 1 atmosphere of pressure (760 mm. of mercury) occupies 22.4 liters.

If a solution is placed in a **semipermeable membrane** (that is, a membrane permeable to water but not to the dissolved substance) and then the membrane is placed in water, it will be found that water will pass through the membrane and dilute the solution. The passage of water through a semipermeable membrane into a solution is called **osmosis.**

A common method of preparing a semipermeable membrane is to take a porous cup and deposit in its pores copper ferrocyanide. This is done by filling a porous cup with a solution of $CuSO_4$ and suspending the cup in a solution of $K_4Fe(CN)_6$. As the two solutions meet in the pores of the cup, they form a precipitate of $Cu_2Fe(CN)_6$. The copper ferrocyanide becomes the membrane, which is given rigidity by the porous cup. If such a semipermeable membrane is filled with a solution containing 1 gram molecular weight of a compound that does not ionize, dissolved in 1 liter of water, the opening is sealed with a manometer, a device for measuring pressure (see Fig. 1), and the cup is placed in water, it will be found that a pressure of 22.4 atmospheres will develop in the cup if the volume is kept constant. If the experiment is repeated with a solution of such concentration that 1 gram molecule is dissolved in 22.4 liters of water, the pressure will be 1 atmosphere. This pressure is called **osmotic pressure** and may be measured by determining the pressure that must be applied to a solution to prevent an increase in volume when the solution is separated from water by a semipermeable membrane.

From the examples just given, it appears that a compound in solution acts very much like a gas with respect to the volume occupied by a gram molecule and the pressure exerted. In physical chemistry a common method for determining the molecular weight of a compound which can be converted into a gas is to measure at a known temperature and pressure the volume occupied by a given weight of the compound when converted into a gas. From this data the weight of the compound necessary to give a volume of gas of 22.4 liters at 0°C. and 760 mm. pressure can be calculated, and the result is the molecular weight of the compound. For example, if 0.1 gram of a pure chemical compound is converted into a gas and the volume of the gas, when measured and reduced to standard conditions, is 20 ml., the molecular weight of the compound may be calculated by solving for X in the following proportion: thus,

$$0.1:X::20:22{,}400$$
$$20X = 2240$$
$$X = 112$$

In a similar manner an osmotic-pressure method may be used for determining the molecular weight of a compound which is soluble in water and does not ionize. All that is necessary is to dissolve a known weight of a compound in a known volume of water and determine the osmotic pressure of the solution. From the data obtained, the weight of the compound which, when dissolved in 1 liter of water, will give an osmotic pressure of 22.4 atmospheres can easily be calculated by simple proportion. The result will be the molecular weight of the compound. For example, if 10 grams of a compound which does not ionize, dissolved in 1 liter of water, gives an osmotic pressure of 2 atmospheres, the molecular weight of the compound may be calculated by solving for X in the following proportion; thus,

$$10:X::2:22.4$$
$$2X = 224$$
$$X = 112$$

Many theories have been advanced to explain osmotic pressure, but none seems to be entirely satisfactory. Osmosis does appear, however, to be related to the attraction which exists between a compound in solution and the solvent. When a compound in solution is separated from water by a semipermeable membrane, water undoubtedly passes both in and out of the membrane. Because of the attraction of the solute for the solvent, water passes in more readily than out, and hence the volume tends to increase on the inside, or, if this is prevented, a pressure develops.

Dialysis. Most membranes are not strictly semipermeable; they allow other simple molecules or ions to pass through them in addition to water. For this reason it is possible to separate smaller simple molecules from larger complex molecules by allowing the simple molecules to diffuse through such a membrane. This process is called **dialysis.**

A good example of dialysis would be the separation of salt from a solution containing albumin and salt. If such a solution were placed in a membrane permeable to salt but not to albumin, and the membrane were placed in water, the salt would pass through the membrane into the water leaving the albumin inside the membrane. If the water on the outside were changed frequently, eventually most of the salt would be removed from the solution inside the membrane.

Since the cells of the body are filled with solutions and since the cell walls are membranes, it is evident that diffusion, osmosis, and dialysis are important factors in life processes. The absorption of food from the intestine, the distribution of food throughout the body, and the elimination of waste products from the cells are due at least in part to these phenomena.

The osmotic pressure of cells can be measured by placing them in a series of solutions of different osmotic pressures. If the osmotic pressure of a solution is greater than that of the cell, water will pass from the cell to the solution, and the cell will shrink. Such a solution is said to be **hypertonic** to the cell, and the shrinkage of the cell is called **plasmolysis.** (See Fig. 2.) If the solution has a lower osmotic pressure than that of the cell, water will pass into the cell from the solution, and the cell will swell. Such a solution is said to be **hypotonic** to the cell, and the swelling of the cell is called **plasmoptysis.** If the solution has the same osmotic pressure as the cell, the cell will neither shrink nor swell, and the solution is said to be **isotonic.** If the osmotic pressure of a solution isotonic to a cell is known, the osmotic pressure of the cell is also known.

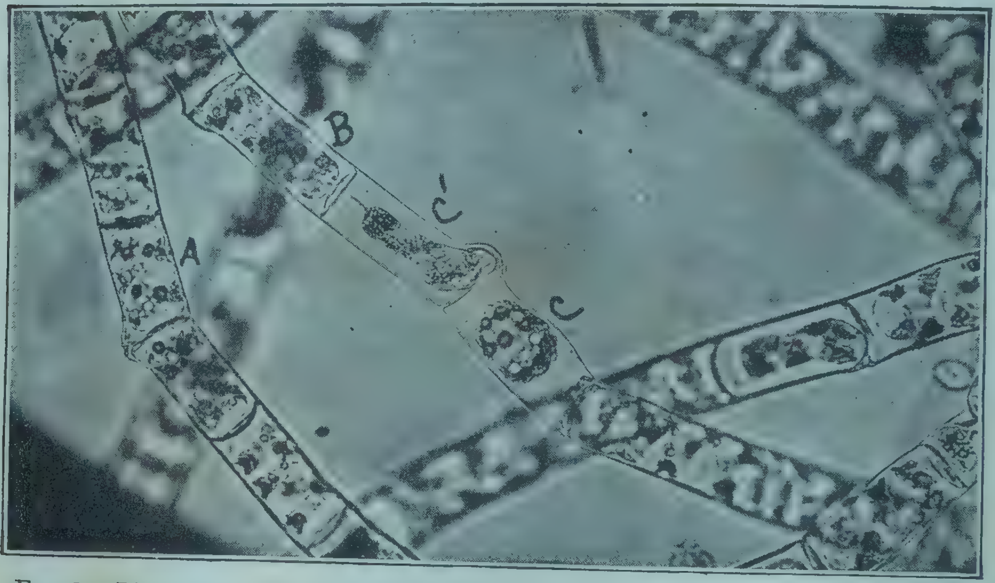


Fig. 2. Plasmolysis of cells of *Spirogyra* in 0.35 *M* sucrose solution. Cell *A* is essentially normal. Cells *B*, *C*, and *C'* show various degrees of plasmolysis. (After Lloyd.) From *Outlines of Biochemistry* by Gortner. John Wiley & Sons, by permission.

If human blood cells are tested in this manner, it is found that their osmotic pressure is about 6.6 atmospheres at 0°C. A solution containing about 0.9 per cent of NaCl has an osmotic pressure of about 6.6 atmospheres at 0°C. and is often spoken of as an **isotonic** or **physiological**

salt solution. In medicine, when it becomes necessary to introduce solutions into the blood stream, it is extremely important to use isotonic solutions. Lack of care in this respect would destroy the delicate membranes of the blood cells, with very serious consequences. Likewise it is important to use isotonic solutions rather than water in applying medication to delicate membranes, such as those in the eye or nasal cavity. Everyone is familiar with the pain associated with getting water in the eyes or in the nose. Isotonic salt solutions, on the other hand, are painless. In giving enemas isotonic salt solutions are preferable to water because they do not injure the delicate membrane lining the intestine.

Depression of the Freezing Point. Another important property of solutions is that a compound in solution lowers the **freezing point** of the solvent. Lowering of the freezing point, like osmotic pressure, is proportional to the amount of the compound dissolved. One gram molecular weight of a compound which does not ionize, dissolved in 1 liter of water, will depress the freezing point 1.86°C. Since a definite relationship exists between the amount of the compound in solution and the osmotic pressure and the depression of the freezing point, it is easily seen that osmotic pressure and freezing-point depression are related to each other. In fact, because of the difficulties of technique involved in determining osmotic pressure directly, the usual method is to determine it indirectly by means of the freezing-point method. Just as osmotic-pressure determinations are used in finding the molecular weights of compounds in solution, so also the depression of the freezing point may be used. For example, if 50 grams of a compound which does not ionize is dissolved in 1 liter of water, and the freezing point is depressed 0.93°C., it is possible to calculate the molecular weight of the compound by solving for X in the following proportion; thus,

$$\begin{aligned} 50:X&::0.93:1.86 \\ 0.93X &= 93 \\ X &= 100 \end{aligned}$$

These data may also be used to calculate the osmotic pressure of the solution by solving for X in the following proportion; thus,

$$\begin{aligned} 0.93:1.86&::X:22.4 \\ 1.86X &= 20.832 \\ X &= 11.2 \end{aligned}$$

This property of substances in solution of depressing the freezing point is of great importance to the biochemist. It places at his disposal a simple and accurate method for determining the osmotic pressure of biological materials, the importance of which has been discussed. It explains why a slight frost may not be a killing frost. Because of the substances in solution, protoplasm will not freeze at the freezing point of pure water. This property of solutions has been applied very practically in the dairy industry to test milk for added water. The **freezing point of milk** has been found to be constant at −0.56°C. If water is added to milk, the freezing point is nearer 0°C. Tables have been prepared which tell with a high degree of accuracy the amounts of water added to milk corresponding to freezing points from 0°C. to −0.56°C.

In explaining why the freezing point of a solution is lower than that of pure water, a theory similar to the one used to account for osmotic pressure may serve. When a solution freezes, pure water separates from the solution. In other words, water is taken away from the dissolved substance, and since the solute has an attraction for the solvent, a lower temperature is necessary to bring about freezing than would be required if water alone were frozen.

Elevation of the Boiling Point. Another property of solutions which is related to osmotic pressure and to the freezing point is the **boiling point.** Since at the boiling point many of the compounds of protoplasm are altered in nature — the coagulation of albumin is an example — the boiling-point method is not often used in dealing with biological materials. However, it will be mentioned briefly. One gram molecule of a compound which does not ionize dissolved in 1 liter of water will elevate the boiling point 0.52°C. Because this value is related to the molecular weight, this method also may be used to determine the molecular weight of a compound and likewise to determine the osmotic pressure and freezing point of a solution.

If 100 grams of a compound which does not ionize is dissolved in 1 liter of water and the boiling point is 100.26°C., the elevation of the boiling point would be 0.26°C. From these data the molecular weight of the compound may be calculated by solving for X in the following proportion; thus,

$$\begin{aligned} 100:X&::0.26:0.52 \\ 0.26X &= 52 \\ X &= 200 \end{aligned}$$

The depression of the freezing point of the solution may be calculated by solving for X in the following proportion; thus,

$$\begin{aligned} 0.26:0.52&::X:1.86 \\ 0.52X &= 0.4836 \\ X &= 0.93 \end{aligned}$$

In a similar manner the osmotic pressure of the solution may be calculated by solving for X in the following proportion; thus,

$$0.26:0.52::X:22.4$$
$$0.52X = 5.824$$
$$X = 11.2$$

The explanation of why the boiling point is elevated by a substance in solution is based on the fact that substances in solution lower the vapor pressure of water. Since the boiling point is the point at which the vapor pressure is equal to the atmospheric pressure, it is evident that a higher temperature will be required for a solution to reach the boiling point than for pure water. It may also be pointed out that, in the boiling of a solution, solvent is being separated from solute. Since, as has been stated, an attraction exists between solvent and solute, the elevation of the boiling point of a solution may be explained as being due to the extra effort necessary to separate the solvent from the solute.

Properties of solutions, such as those just discussed, that depend upon the number of particles per unit volume rather than on chemical properties are called **colligative** properties.

In the above discussion of osmotic pressure, depression of the freezing point, and elevation of the boiling point, we have been referring to solutions of compounds that do not ionize. It should be pointed out that in solutions of compounds that do ionize greater deviations from the behavior of pure water are obtained for a given concentration of a compound, since ions behave as molecules with respect to these properties of solutions. It should also be mentioned that certain elements, such as oxygen, in solution would behave as compounds.

Surface Tension. Another property of solutions of biological importance is that substances in solution alter the **surface tension** of the solvent. Since surface phenomena are so important in life processes, it will be well to refresh our minds as to the meaning of surface tension. Everyone is familiar with the fact that a needle may be made to float if it is carefully placed on the surface of water. The water gives the appearance of being covered with a thin, elastic surface film. This film is due to a contraction of the liquid at its surface caused by surface tension, and is responsible for the resistance of a surface film to rupture. Surface tension accounts for many other interesting phenomena such as the ability of small insects to walk on water without becoming wet, the tendency for drops of liquid to be spherical, the rise of water in a capillary tube, and the movement of water in a blotter.

In the center of a body of liquid the molecules are attracted equally in all directions by other, similar molecules. (See Fig. 3.) At the surface the molecules are attracted more toward the center of the liquid than they are by the molecules of air above the liquid; hence it is assumed that at the surface the molecules are more compact. This concentration of molecules at the surface may be sufficient to give the surface layer some of the properties of a solid and in this way may account for the surface film.

Surface tension exists not only at the boundary between a liquid and air but also at any point a liquid is in contact with another liquid or a solid. It will be evident, then, that the amount of surface existing in protoplasm, which is filled with particles of colloidal size, is enormous and that surface tension is a factor to be reckoned with in biochemistry.

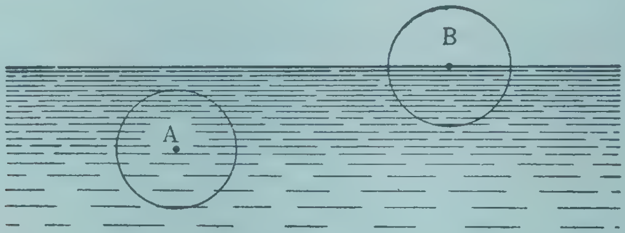


FIG. 3. Forces of molecular attraction acting on molecules. The circles represent the field of attraction on molecules at their centers. In *A*, within the liquid, the force is equal in all directions. In *B*, at the surface, the force is greater downward than upward because of the higher concentration of molecules in the liquid than in the vapor phase.

It should be noted that the contraction at a surface requires energy and that surface tension involves energy. Many of the energy changes which take place in protoplasm may be accounted for by changes in surface tension. If surface tension is reduced in a system, the energy liberated becomes available for other purposes.

Substances in solution alter the surface tension of the solvent. Often, the inorganic salts increase it, whereas substances like fat, soap, and bile salts decrease it. A well-known principle states that the amount of free energy in a system will decrease if possible. Since surface tension involves such a form of energy, it is not surprising to find that substances which lower the surface tension of water concentrate in the surface film so as to lower the surface energy as much as possible. This concentration at a surface is called **adsorption.** Substances that increase surface tension tend to stay away from the surface film and concentrate in the interior of the liquid in order to increase the surface energy as little as possible. This decrease in concentration in the surface film is spoken of as **negative adsorption.** Adsorption and negative adsorption are important biological phenomena.

Hydrogen-ion Concentration

Acidity and alkalinity, often referred to as the reaction of a solution, are extremely important factors in the proper functioning of living organisms. If the reaction of our blood changes only slightly, acidosis or alkalosis will result, ending in death if allowed to go too far. In bacteriology great care must be taken to regulate the reaction of a medium in order to make it suitable for the growth of a particular microorganism. In order to understand modern biology, it is quite necessary for the student to know what is meant by **hydrogen-ion concentration,** and to do this he should be familiar with some of the theory back of it.

Quantity Factor of Acidity. An **acid** may be defined as any substance that gives hydrogen ions when in solution. When we speak of the concentration of an acid, we may be considering either one of two factors, namely, the quantity of acid in a given volume or the intensity of acidity. In that part of quantitative analysis which deals with acidimetry and alkalimetry we are interested in the amount of acid present in a given volume of solution. We do not care whether the acid in solution is in the molecular or the ionic form. A solution which contains 1 gram atom, that is, 1.008 grams, of acid hydrogen per liter is called a **normal solution of an acid.**

A **base** may be defined as a substance which will give hydroxyl ions when in solution. When a base neutralizes an acid, the H ion of the acid combines with the OH ion of the base to form H_2O. Hence an OH ion in a base is equivalent to an H ion in an acid. A **normal solution of a base** contains 1 gram radical of ionizable OH, that is, 17.008 grams per liter. Normal solutions of acids and bases are equivalent; 1 ml. of a normal solution of an acid will exactly neutralize 1 ml. of a normal solution of a base.

To make a normal solution of HCl, 1 gram molecule of HCl, or 36.458 grams per liter of solution, is needed in order to supply 1.008 grams of H. In like manner 40.008 grams of NaOH is required to obtain 17.008 grams of OH for 1 liter of normal solution. To make a normal solution of H_2SO_4 ½ gram molecule of this acid must be dissolved in a liter, since in 1 gram molecule of H_2SO_4 there are 2 gram atoms of H. The same principle would apply for bases, such as $Ba(OH)_2$, where there are 2 gram radicals of OH per gram molecule.

In practice an exactly normal solution is rarely used; instead, carefully standardized solutions whose concentrations are designated by normality factors are employed. A **normality factor** is a number by which to multiply in order to convert any number of milliliters of solution into milliliters of normal solution. If 100 ml. of a solution whose normality factor is 0.1252 is used, only a very simple calculation is needed to arrive at the conclusion that this is equivalent to 12.52 ml. of normal solution. For brevity the letter N is usually used for normal, and the above solution may be designated 0.1252 N. A tenth normal solution is often written 0.1 N or $N/10$. Any solution the exact concentration of which is known is called a **standard solution.**

Determination of the quantity of acid in a biological fluid such as urine is often desirable. This procedure involves adding to a definite volume of the urine a solution of base whose normality is known. The solution of base is added from a burette in order that the volume of alkali required for neutralization may be accurately determined. The process is called **titration.** Phenolphthalein is used as an indicator to tell when the acid in the urine has been neutralized. Results are expressed as the number of milliliters of 0.1 N base necessary to neutralize a 24-hour sample of the urine. In this way the quantity of acid eliminated per day can easily be calculated. This is known as **titratable acidity,** and it should be noted that it is a measure of the quantity of acid present, not of the intensity of the acidity.

Intensity Factor of Acidity. We will next consider the intensity of acidity. When an acid is dissolved in water, some of the molecules dissociate into hydrogen ions and acid radical ions. The intensity of an acid depends upon the degree of this dissociation or rather upon the concentration of hydrogen ions. From the intensity standpoint we call an acid normal if there is 1.008 grams of ionic hydrogen per liter. Such a solution may be many times normal from the standpoint of quantity of acid present.

In order to comprehend hydrogen-ion concentration the **law of mass action,** which states that the speed of a chemical reaction is proportional to the molecular concentration of the reacting substances, must first be understood. In the reversible reaction

$$\underset{\substack{\text{Acetic}\\ \text{acid}\\ a}}{CH_3COOH} + \underset{\substack{\text{Ethyl}\\ \text{alcohol}\\ b}}{C_2H_5OH} \rightleftharpoons \underset{\substack{\text{Ethyl}\\ \text{acetate}\\ c}}{CH_3COOC_2H_5} + \underset{\substack{\text{Water}\\ d}}{H_2O}$$

the speed from left to right is proportional to the concentrations of a and b. In algebra we are taught that if a thing is proportional to two or more things it is proportional to their product. Hence the speed from left to right is proportional to $[a] \times [b] \times k$, where k is a constant whose value depends on such conditions as temperature. (In the above expression and following equations a factor in brackets means the concentration of that factor.) In like manner the speed of the reaction from right to left is proportional to $[c] \times [d] \times k'$. When the reaction has proceeded to equilibrium, the speeds in each direction must be equal. Hence

$$[a] \times [b] \times k = [c] \times [d] \times k'$$

from which we may derive the following equation:

$$\frac{[c] \times [d]}{[a] \times [b]} = \frac{k}{k'}$$

Since a constant divided by a constant is equal to a constant, we have

$$\frac{[c] \times [d]}{[a] \times [b]} = K$$

This K is called the **equilibrium constant.**

In water we find that some of the molecules ionize thus:

$$H_2O \rightleftharpoons H^+ + OH^-$$

If we apply our definitions of acid and base to water, we find that water is an acid and at the same time a base. Since a molecule of water gives an equal number of H^+ and OH^-, we say that it is neutral; that is, it is just as strong an acid as it is a base. If a substance which gives H^+ in solution is added to water, there will be more H^+ than OH^-, and hence we say that the solution is acidic.

If we apply the law of mass action to the last equation as we did to the first, we find

$$\frac{[H^+] \times [OH^-]}{[H_2O]} = \frac{k}{k'} = K$$

Since water ionizes only slightly, the value of the concentration of H_2O, for all practical purposes, may be considered a constant, which we may call w. Hence the equation becomes

$$\frac{[H^-] \times [OH^-]}{w} = K$$

or $$[H^+] \times [OH^-] = K \times w$$

or $$[H^+] \times [OH^-] = Kw \quad \text{or} \quad K_w$$

Kw, the ionization constant for water, is usually referred to as K_w.

Methods are available for determining the H^+ concentration of pure water. It has been found that pure water is 1/10,000,000 N with respect to H^+ at 22°C. Mathematically the fraction 1/10,000,000 is usually expressed as 10^{-7}. We then say that the H^+ concentration of pure water is 10^{-7} N. Since in pure water the concentration of H^+ and the concentration of OH^- are equal, the concentration of each must be 10^{-7}, and K_w is then 10^{-14}..

$$\begin{array}{ccc} [H^+] \times & [OH^-] = & K_w \\ 10^{-7} & 10^{-7} & 10^{-14} \end{array}$$

Pure water, then, may be looked upon as an acid which is 10^{-7} N with respect to H^+ and also as a base which is 10^{-7} N with respect to OH^-.

If some acid is added to water, the concentration of H^+ will increase. Let us suppose that the solution becomes 1/100 N with respect to H^+. The concentration of H^+ of such a solution is 10^{-2} N. From our original equation we know that $[H^+] \times [OH^-] = 10^{-14}$. If we know that the concentration of H^+ is 10^{-2}, then we also know that the concentration of OH^- is 10^{-12}, since $10^{-2} \times 10^{-12} = 10^{-14}$. If we add a base to water, we increase the concentration of OH^- above that of pure water. Let us suppose that we add enough base to water to give a solution which is $N/1000$ with respect to OH^-. The concentration of OH^- will then be 10^{-3} N. Knowing this, we can easily calculate the concentration of H^+. It is 10^{-11}, because $10^{-11} \times 10^{-3} = 10^{-14}$.

Thus it is seen that, if the concentration of H^+ is known, the concentration of OH^- is also known. It thus becomes possible to express degrees of alkalinity in terms of H^+ concentration. If the negative exponent of 10 is 7, the solution is neutral; if less than 7, acid; and if more than 7, alkaline.

Since in expressing H^+ concentration the expression 10^- is always used, Sörensen suggested leaving 10^- out and using only the numerical value of the negative exponent. He called this the ***p*H value.** In the expression pH, p means power (negative exponent), and H, the hydrogen-ion concentration. Mathematically, pH is defined as the logarithm of the reciprocal of the hydrogen-ion concentration. If a solution had a H^+ concentration of 10^{-6}, Sörensen would have said that the pH was 6. At the present time the Sörensen system is ordinarily used, and it is customary to speak of pH values rather than H^+ concentrations. The Sörensen pH scale runs from 0 to 14 and covers the range from normal acid to normal base. A solution with a pH of less than 7 is acid, and one with more than 7 is basic. By means of the pH scale any degree of acidity or alkalinity found in biological materials can be expressed.

From what has been said so far, it might be concluded that only whole numbers are used in expressing H^+ concentrations as pH. This is not so, and most certainly should not be so, since one of two solutions differing from each other by 1 pH is ten times as strong an acid as the other. For example, a solution with a pH of 2 is 0.01 N, whereas one with a pH of 3 is 0.001 N with respect to H^+. In other words, the first solution is ten times as concentrated an acid as the second. It is very important to realize the great differences in degrees of acidity which as little as 1 *p*H means.

Determination of Hydrogen-ion Concentration. Several methods are available for the determination of the *p*H of a solution. These may be classified under two main headings, namely, electrometric and colorimetric methods. The electrometric methods are the more accurate but require more expensive equipment than the colorimetric methods. Where extreme accuracy is not required the latter methods are usually employed.

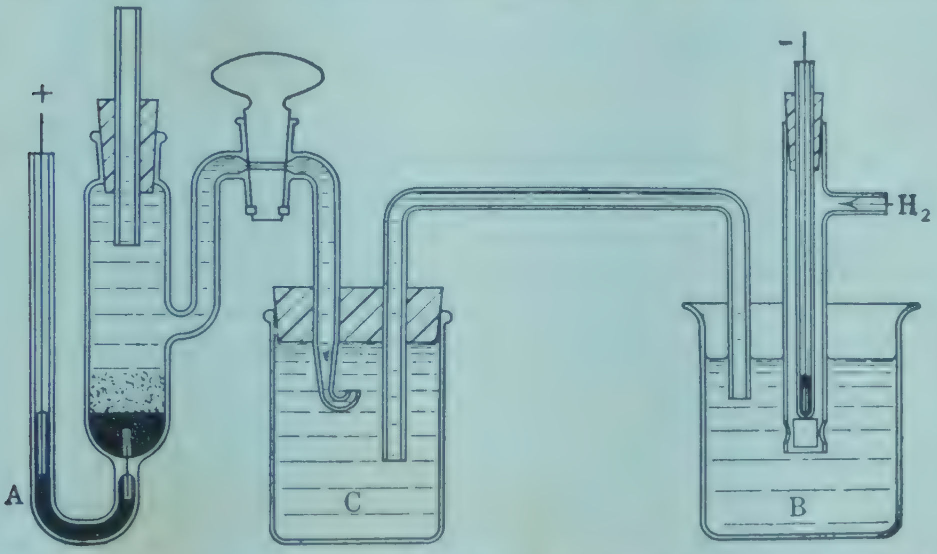


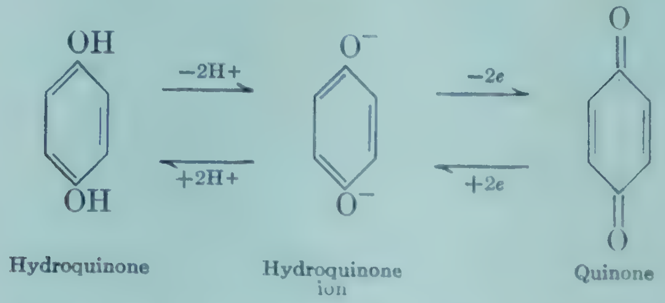
FIG. 4. Apparatus for measuring hydrogen-ion concentration electrically. *A* is a calomel electrode containing mercury covered with HgCl and a KCl solution; *B* is the unknown solution containing the hydrogen electrode; *C* is a saturated solution of KCl which acts as a salt bridge. The two electrodes are connected to a potentiometer for measuring difference of potential. Courtesy of Leeds and Northrup Co.

Electrometric Methods. HYDROGEN ELECTRODE METHOD. There are several electrometric methods available for determining *p*H. The one which was first used, which covers the entire *p*H range, and which is the most easily understood, is a method that makes use of a hydrogen electrode. (See Fig. 4.) If a platinum electrode is coated with platinum black and then saturated with hydrogen gas, the platinum black will absorb hydrogen on the surface of the electrode. The electrode then behaves as though it were made of hydrogen. It will be recalled that in many reactions hydrogen acts like a metal. If a platinum electrode coated with platinum black is placed in a solution containing hydrogen ions and saturated with hydrogen gas, hydrogen from the electrode will go into solution, forming hydrogen ions, which have a positive charge of electricity. The positive charge comes from the electrode, leaving it negative. The number of H^+ going into solution is inversely proportional to the number already present in the solution, so that the negative charge on the electrode is dependent on the H^+ concentration of the solution. In other words, the electrical potential of the hydrogen electrode is a function of the H^+ concentration of the solution in which it is placed. If such a hydrogen electrode is connected with another electrode, whose electrical potential is known, it is easy to measure the difference of potential between these two electrodes by means of a potentiometer, an instrument for measuring differences in potential.

In practice a **calomel electrode** is commonly used as the electrode of known potential. This is made of mercury, calomel, and a potassium chloride solution. The potassium chloride solution must be of definite concentration; 0.1 *N*, 1 *N*, 3.5 *N*, and saturated solutions are commonly employed. Such electrodes have a very definite and constant potential for any given concentration of potassium chloride. It is very important in using a calomel electrode to know from what concentration of potassium chloride solution the electrode was prepared. Tables are available which give *p*H values corresponding to differences of potential between the hydrogen electrode and the four types of calomel electrodes just mentioned.

QUINHYDRONE ELECTRODE METHOD. A second electrometric method for determining hydrogen-ion concentration which has several advantages over the hydrogen-electrode method employs the quinhydrone electrode. The quinhydrone electrode is much simpler to operate than the hydrogen electrode. It avoids the necessity of coating the electrode with platinum black and saturating it with hydrogen gas. Two of its disadvantages are that it is not applicable for measuring *p*H values above 8.5 and that it is very sensitive to temperature changes. In this method the solution to be tested is saturated with quinhydrone by adding a small quantity of the powdered substance. A gold or platinum electrode, which has not been coated with platinum black, is then placed in the solution, and the difference in potential between it and a standard calomel electrode is measured by means of a potentiometer. The *p*H may be obtained by consulting a table which converts potentiometer readings into *p*H values.

Quinhydrone is a compound consisting of equimolecular quantities of quinone and hydroquinone. A saturated solution of quinhydrone contains equimolecular quantities of quinone and hydroquinone. Some of the hydroquinone molecules ionize to form double negative hydroquinone ions and positive hydrogen ions. The hydroquinone ion easily loses two electrons, forming quinone.



When an electrode is placed in a solution containing quinhydrone, the hydroquinone ions tend to give up their electrons to the electrode, making it negative. The degree of negativity or potential of the electrode is therefore dependent on the concentration of the hydroquinone ion in the solution. As might be expected from the foregoing equation, the degree of ionization of hydroquinone is determined by the hydrogen-ion concentration of the solution; in other words, the concentration of hydroquinone ions is determined by the hydrogen-ion concentration. Acid added to the solution depresses the ionization of the hydroquinone and therefore reduces the concentration of hydroquinone ions. Since in a saturated solution of quinhydrone the concentration of hydroquinone and quinone remains constant, we find that in the equation only the hydroquinone ion is altered by a change in hydrogen-ion concentration of the solution. Thus it is clear that, if the potential of a quinhydrone electrode is determined by the concentration of hydroquinone ions, it is also determined by the hydrogen-ion concentration of the solution. The potential of the quinhydrone electrode therefore becomes a measure of the hydrogen-ion concentration.

GLASS ELECTRODE METHOD. A third electrometric method for the determination of hydrogen-ion concentration which is being used a great deal at the present time utilizes the glass electrode. One type of glass electrode consists of a thin bulb of glass filled with a standard solution of an electrolyte, such as 0.1 *N* HCl. When this electrode is placed in the solution to be tested, a difference of potential develops between the solution inside the electrode and that on the outside. The value of this difference depends upon the hydrogen-ion concentration of the solution outside the electrode, since that inside the electrode remains constant. The potential of the glass electrode is determined in the same way as that of the hydrogen or quinhydrone electrode, namely, by measuring the difference of potential between it and a calomel electrode. Because of the high resistance of the glass membrane separating the solutions inside and outside the glass electrode, vacuum-tube amplifiers are introduced into the potentiometer circuit in order to permit taking a reading. Instruments are on the market which read *p*H values directly.

The advantage of the glass electrode over the hydrogen electrode is that no hydrogen is necessary, and therefore the glass electrode can be used on solutions containing dissolved carbon dioxide. Since carbon dioxide dissolved in water forms carbonic acid it is obvious that its removal by bubbling hydrogen through the solution would alter the *p*H of the solution. The advantage over the quinhydrone electrode is that the solution to be tested is not contaminated with quinhydrone and may be used for other purposes after the *p*H value is determined. The method is accurate up to a *p*H of 10 or even higher with special electrodes.

Colorimetric Method. The colorimetric method for the determination of *p*H makes use of the fact that certain dyes change color at very definite *p*H values. Such dyes are often called **indicators** because, when added to a solution, they indicate whether it is acidic or basic; and, since they change color when the reaction changes, they are used in acid and base titrations to tell when an acid or a base is neutralized. The various indicators change color at different *p*H values. Methyl orange changes color at a *p*H of 2.9 to 4.0, Congo red between 3.0 and 5.0, sodium alizarin sulfonate between 5.5 and 6.8, litmus at about 7, and phenolphthalein between 8.3 and 10.0. If a drop of indicator solution is added to a solution of unknown *p*H, it is possible to tell whether its *p*H is greater or less than that at which the indicator changes color. For example, to determine the approximate *p*H of saliva, a drop of phenolphthalein solution may be added to some saliva in a test tube. If the solution remains colorless, the *p*H of the saliva is less than 8.3. To another tube of saliva litmus may be added. If the color is blue, the *p*H is greater than 7. In other words, by these simple tests, it is possible to determine that the *p*H of the saliva is between 7 and 8.3.

BUFFERS. For a more accurate determination of *p*H by the colorimetric method, a series of buffer solutions of known *pH* values are utilized. A buffer solution is a solution of a weak acid or base together with its salt. The term weak or strong, when applied to an acid or a base, means that it gives few or many, respectively, hydrogen or hydroxyl ions in dilute solution. A buffer solution tends to retain its *p*H value upon the addition of small amounts of acid or base. A good example of a buffer solution in which the *p*H value falls on the acid side of neutrality is a solution of sodium acetate and acetic acid in water. If a small amount of HCl is added, it will react with the sodium acetate, forming NaCl and acetic acid. The acetic acid, being weak, will affect the hydrogen-ion concentration very little. If HCl is added to water, there will be a decided change in the hydrogen-ion concentration of the water, because most of the HCl molecules will ionize. If NaOH is added to a sodium acetate-acetic acid buffer solution, some of the acetic acid will be neutralized, forming more sodium acetate. The removal of some of the acetic acid from the buffer solution will affect the hydrogen-ion concentration very little, because most of the acetic acid in the buffer solution is in the molecular form.

If to an equal volume of each of a series of buffer solutions, covering the range in which the *p*H of the unknown is expected to fall, is added a definite quantity of an indicator which changes color over that range, a series of colors ranging from the color of the indicator in acid to that of the indicator in base will be obtained. If to an equal volume of the unknown solution is added the same quantity of the indicator as was used with the buffer solutions, a color will develop which should match the color in one of the known tubes. The *p*H of the unknown solution is then equal to the *p*H of the known solution which gives the same color with the indicator.

From what has been said it should not be concluded that buffers are only important for the determination of *p*H. Body tissues and most secretions of the body are slighly alkaline in reaction. Blood, for example, normally has a *p*H of about 7.35. If this *p*H changes by only a few tenths death may result. To maintain a constant *p*H in the blood several buffers are present such as $NaHCO_3$ and H_2CO_3 and NaH_2PO_4 and Na_2HPO_4. These and other buffer systems in the blood will be discussed in the chapter on blood.

INDICATORS. It has been pointed out that indicators change color at different *p*H values. Therefore, in titrating an acid against a base the results obtained will vary, depending upon the choice of indicator. Quite different results may be expected in an acid-base titration with methyl orange, which changes color at a *p*H of 2.9 to 4.0, from those obtained with phenolphthalein, which changes color at a *p*H of 8.3 to 10.0. The choice of the indicator for an acid-base titration is therefore very important, when titrating weak acids or bases. A dilute solution of a weak acid or base may contain many molecules capable of being neutralized, but very few hydrogen or hydroxyl ions. Thus between the *p*H range for methyl orange and phenolphthalein the quantity of acid or base to be titrated may be large. In titrating a weak acid, such as acetic, with NaOH, methyl orange, which changes color at a *p*H of 2.9 to 4.0, should never be used, because acetic acid is so poorly ionized that at a *p*H of 4.0 considerable acid will still remain unneutralized. Phenolphthalein may be used, because it changes color on the alkaline side of *p*H 7. Since NaOH is highly ionized in solution, a very small amount of it, added to the solution after the acetic acid is neutralized, will throw the *p*H beyond 8.3, thus changing the color of phenolphthalein. In general, in titrating a weak acid against a strong base, an indicator that changes color on the alkaline side of neutrality should be chosen.

In like manner, in titrating a weak base, like NH_4OH, against a strong acid, phenolphthalein should never be used as an indicator; one should be chosen that changes color on the acid side of neutrality. Phenolphthalein will change color while there is still considerable NH_4OH in the solution unneutralized. If an indicator like methyl orange is used, however, all the NH_4OH will be neutralized before it changes color. A very small amount of a strong acid which ionizes readily will change the *p*H of the solution beyond the point where methyl orange changes color after all the NH_4OH is neutralized. In general, in titrating a weak base against a strong acid an indicator that changes color on the acid side of neutrality should be selected.

If a strong base is titrated against a strong acid, the choice of an indicator is not so important. However, it should be kept in mind that phenolphthalein is sensitive to even such a weak acid as H_2CO_3, and hence it cannot be used where carbonates are present unless the CO_2 is removed by boiling.

Another factor to bear in mind in titrating weak acids and bases against strong bases and acids is that the salts which result do not form neutral solutions in water. Let us take, for example, sodium acetate, which is the salt of a strong base and a weak acid. In aqueous solution this salt tends to hydrolyze thus:

$$CH_3COONa + H_2O \rightleftharpoons CH_3COOH + NaOH$$

Since CH_3COOH is a weak acid, it tends to remain in the molecular form, and very few acetate and hydrogen ions appear. On the other hand, NaOH is a strong base, which means that it has a strong tendency to ionize and give Na^+ and OH^-. The result is that a solution of sodium acetate produces an excess of OH ions, so that the *p*H of the solution is greater than 7. In order to titrate acetic acid with sodium hydroxide to the point where an equivalent amount of sodium hydroxide has been added, it is necessary to titrate to the *p*H which sodium acetate will give in solution and not to a *p*H of 7. This is another reason for using phenolphthalein in this titration rather than an indicator which changes color at a *p*H of less than 7.

A similar line of reasoning reveals the fact that the salt of a weak base and a strong acid gives a solution whose *p*H is less than 7, and

TABLE 1

COMMON INDICATORS

Indicator		Color		pH Range	Preparation for Use
Common Name	Chemical Name	Acid	Base		
Thymol blue	Thymolsulfonephthalein	Red	Yellow	1.2–2.8 (acid range) 8.0–9.6 (alkali range)	0.1% water solution of sodium salt
Methyl orange	Dimethylaminoazobenzenesulfonate	Red	Yellow	2.9–4.0	0.1% solution in 50% alcohol
Congo red	Sodium tetrazodiphenylnaphthionate	Blue	Red	3.0–5.0	0.5% alcoholic solution
Methyl red	*o*-Carboxybenzeneazodimethyl aniline	Red	Yellow	4.4–6.0	0.05% alcoholic solution
Alizarin red	Sodium alizarin sulfonate	Yellow	Purple	5.5–6.8	1.0% water solution of sodium salt
Bromothymol blue	Dibromothymolsulfonephthalein	Yellow	Blue	6.0–7.6	0.1% water solution of sodium salt
Litmus	....	Red	Blue	About 7.0	Paper
Cresol red	*o*-Cresolsulfonephthalein	Yellow	Red	7.2–8.8	0.1% solution in 50% alcohol
Phenolphthalein	Dihydroxyphthalophenone	Colorless	Red	8.3–10.0	1.0% solution in 50% alcohol
Cresolphthalein	*o*-Cresolphthalein	Colorless	Red	8.0–10.0	0.5% alcoholic solution

therefore an indicator for titrating a weak base against a strong acid should change color on the acid side of neutrality.

In view of what has been said, it should be quite obvious that in preparing standard solutions of acids or bases the same indicator should be chosen for the standardization as will be used when these solutions are employed in subsequent titrations.

The Colloidal State

The science of colloid chemistry was founded about 1861, when Thomas Graham published a summary of his work on the diffusion of substances in solution through membranes. He found that substances which crystallized readily diffused rapidly, whereas substances which did not form crystals did not diffuse. This fact led him to classify substances into two groups, namely, **crystalloids** and **colloids.** The word colloid, meaning gluelike, was selected because glue is a typical substance that does not diffuse.

It is believed that the failure of colloids to diffuse is due to the size of the particles of the dissolved substances. We now know that it is possible to control the size of the particles of almost any substance in solution by proper methods so that they will not pass through a membrane, and hence substances might be classed as colloids even though they ordinarily exist in crystalline form. Likewise, a typical colloidal substance like egg albumin has been prepared in a crystalline form which, when dispersed in water, will not diffuse through a membrane.

Solution, Colloidal Solution, and Suspension. According to current views, it is not strictly correct to speak of a colloid, because a colloid is not a kind of matter, but rather a state of subdivision of matter. It is much better to speak of the **colloidal state of matter** than to speak of a colloid. If some finely ground substance is placed in water, one of three things may happen. First, it may form a **true solution.** In this case the material probably is broken up into its molecules or ions, and we say that we have a molecular or ionic dispersion of the substance in water. Arbitrarily we have set a limit to the size of particles in true solution as being not larger than one-millionth of a millimeter in diameter. Often 1/1,000,000 mm. is spoken of as a millimicron ($m\mu$); 1 millimicron is 1/1000 of a micron (μ), the latter being 1/1000 mm.

The second possibility is that the substance may form a **colloidal solution.** Arbitrarily we say that colloidal particles range in size from 1/1,000,000 mm. to 1/10,000 mm., or in the other terminology, from 1 $m\mu$ to 0.1 μ. The third possibility is that the particles will be larger

TABLE 2

A COMPARISON OF TRUE SOLUTIONS, COLLOIDAL SOLUTIONS, AND SUSPENSIONS

	True Solutions	Colloidal Solutions	Suspensions
1. Size of particles	Less than 1 mμ	From 1 mμ to 0.1 μ	Over 0.1 μ
2. Diffusibility and filterability	Will pass through membranes and filters	Will pass through filters but not through membranes	Will not pass through either filters or membranes
3. Visibility	Invisible	Ultramicroscopic	Microscopic
4. Motion	Molecular movement	Brownian movement	Slow Brownian and gravitational movement
5. Osmotic pressure	High	Low	None
6. Optical properties	Transparent	Tyndall phenomenon (in suspensoid systems)	Opaque

than 0.1 μ; this mixture is called a **suspension,** because the particles will settle out on standing.

Considering true solution, colloidal solution, and suspension further, we find that in a true solution the particles will pass through a membrane and a filter; in a colloidal solution the particles will pass through a filter but not through a membrane; and in a suspension the particles of suspended material will not pass through either a filter or a membrane.

From the standpoint of visibility we find that particles in true solution are invisible even with the most powerful ultramicroscope. Particles in colloidal solution are **ultramicroscopic,** and particles in suspension are visible with an ordinary microscope or with the naked eye.

In true solution the ultimate particles, which are probably the molecules or ions, display what is known as molecular motion; in colloidal solution the particles display a peculiar type of motion known as **Brownian movement;** in suspension the particles may exhibit slow Brownian movement but will settle to the bottom of the vessel owing to the force of gravity.

Since osmotic pressure is dependent on the number of particles in a given volume, we find that osmotic pressure is high in true solutions and low in colloidal solutions. Suspensions show no osmotic pressure.

If a beam of light is passed through a true solution, the whole solution lights up and appears transparent. In many colloidal solutions the space traversed by the beam of light takes on a hazy appearance due to the reflection of the light by the colloidal particles. This prop-

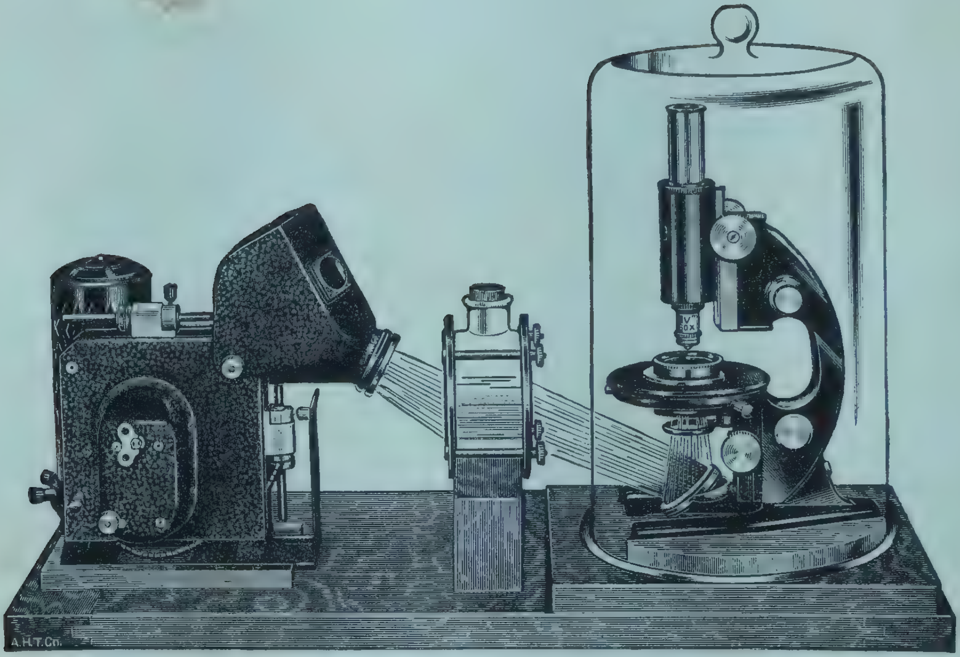


Fig. 5. Zeiss cardioid ultramicroscope assembly.

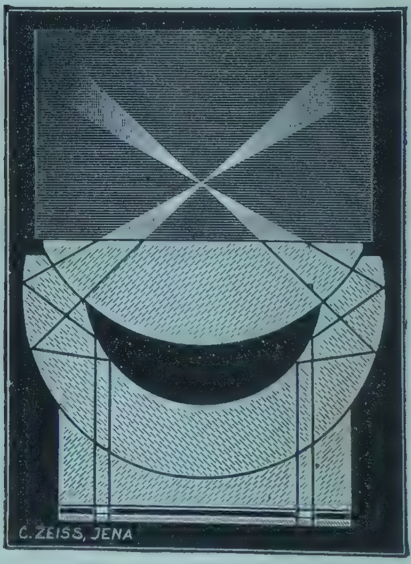


Fig. 6. The path of light rays through a cardioid condenser. The microscope is focused on the point where the light rays cross above the condenser. From *Outlines of Biochemistry* by Gortner, John Wiley & Sons, by permission.

erty of colloidal solutions is spoken of as the **Tyndall phenomenon.** A colloidal solution, like a true solution, appears transparent by transmitted light, but may give the appearance of a suspension by reflected light. Suspensions are opaque.

Table 2 summarizes what has just been said about true solutions, colloidal solutions, and suspensions.

In the above discussion we have used the terms ultramicroscopic and Brownian movement, which possibly need explanation.

Ultramicroscope. By means of the ultramicroscope particles can be detected which are so small that they cannot be seen with even the oil-immersion objective of an ordinary microscope. Everyone is familiar with the fact that the air in a room is filled with dust particles which become visible if a strong beam of light comes into a dark room through a small hole in a curtain. Under these conditions we see particles which are too small to be detected under ordinary circumstances. The particles become visible to us because of the light reflected from them. An ultramicroscope makes use of the same principle on a microscopic scale. (See Figs. 5 and 6.) One type of ultramicroscope consists of an ordinary microscope with a special condenser, which brings to a point beams of light in a microscope field in which the liquid under

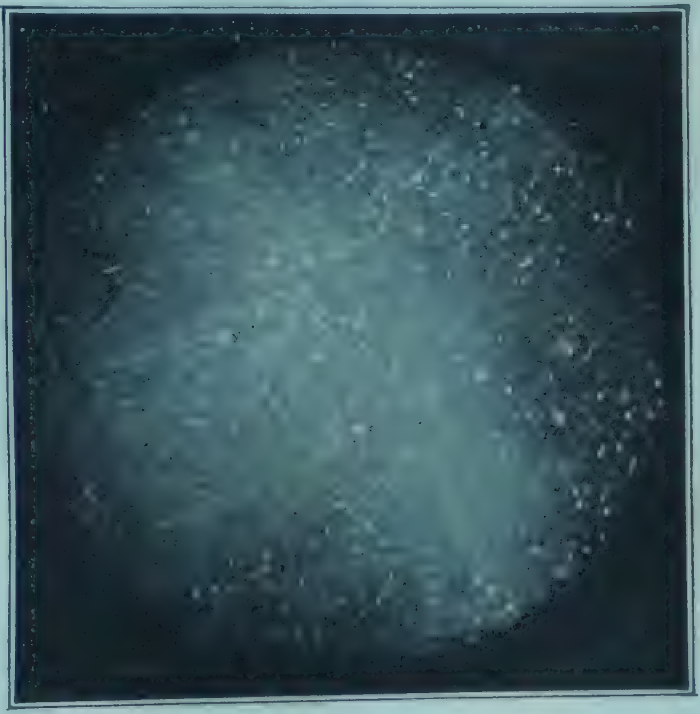


FIG. 7. Photomicrograph of a red gold sol under an ultramicroscope. From *Outlines of Biochemistry* by Gortner, John Wiley & Sons, by permission.

examination may be placed. Beneath the microscope field is a black background which aids in bringing out the reflected light from the colloidal particles. Such a condenser is called a **dark-field condenser.** Under the ultramicroscope the actual colloidal particles are not visible; only bright spots which are the reflected light from these particles can be seen. (See Fig. 7.) It should be mentioned that not all colloids are visible under the ultramicroscope. For ultramicroscope demonstrations red gold preparations are especially satisfactory; dilute colloidal solutions of egg albumin show few or no bright spots. As a rule, suspensoid colloids are more satisfactory than emulsoid colloids for this purpose.

Brownian Movement. If a colloidal solution of gold is observed under an ultramicroscope, it is noted that the particles do not remain still but are in a constant state of rapid vibration. Careful observation reveals that these particles do not move across the microscope field but tend to remain in a rather definite position. Similar motion was first noted in pollen grains by Robert Brown. For this reason the phenomenon has been called Brownian movement. Bacteriologists have noted Brownian movement in hanging drops of bacteria. A beginner often mistakes such movement for true motility, which is possessed by some bacteria.

In summary we may say that the colloidal state exists in a solution when the dispersed particles are so large that they will not pass through a membrane and still so small that they will not settle out.

Definition of Terms. In discussing the colloidal state certain terms must be defined. In the first place, it is necessary to distinguish between the substance in colloidal solution and the solvent. The terms solute and solvent are not used; instead we call the colloidal particles the **dispersed phase** of the colloidal system, and the liquid in which the particles are dispersed the **dispersion medium.**

If the dispersed phase has no affinity for the dispersion medium and does not tend to go into true solution, we call the resulting colloidal system a **suspensoid.** This name indicates its similarity to a suspension. Sometimes a suspensoid is spoken of as a **lyophobic system** to indicate the lack of affinity of the dispersed phase for the dispersion medium. If the dispersion medium is water, such a system is said to be **hydrophobic.** A good example of this type of colloidal system is a colloidal solution of gold.

If the dispersed phase has an affinity for the dispersion medium and tends to take it up and to swell, the system is spoken of as an **emulsoid.** This name indicates its similarity to an emulsion, in which we have a liquid dispersed in a liquid. Such a system is also called a **lyophilic**

system to indicate the attraction of the colloidal particles for the dispersion medium. If the dispersion medium is water, such a system is said to be **hydrophilic.** A good example of an emulsoid is a colloidal solution of gelatin.

Colloidal systems, especially emulsoids, may exist in two forms. A colloidal system that has the properties of a liquid and may be poured from one vessel to another is called a **sol.** A colloidal system that takes the form of a jelly and has many of the properties of a solid is called a **gel.**

The most common colloidal systems are those in which water is the dispersion medium. It should be noted in passing that any liquid may be a dispersion medium. It should also be pointed out that we may have colloidal dispersions of solids, liquids, or gases in solids, liquids, or gases, with the exception of the dispersion of a gas in a gas. For example, smoke may be considered a colloidal dispersion of a solid in a gas, and a fog may be considered a colloidal dispersion of a liquid in a gas. Although the colloidal systems suggested are very interesting and much could be said about them, those in which water is the dispersion medium are the important ones from the biological standpoint.

Charge on Colloidal Particles. It has been shown that in a colloidal system the dispersed particles are so small that they will not settle out, and because of this fact the system is distinguished from a suspension and an emulsion. It may be asked why the particles do not settle out. One explanation is that in a colloidal system the particles are charged electrically. The charge may be either negative or positive. In a given colloidal system all particles have like electric charges and hence tend to remain as far away from one another as possible. As a result, the colloidal particles distribute themselves uniformly throughout the liquid in which they are dispersed.

Electrophoresis. It is possible by a simple experiment to determine the sign of the charge on a colloidal particle. If a colloidal solution is put in the bottom of a U tube, water is carefully added in each arm of the tube, and wires connected to a battery of high voltage are placed in the arms of the tubes, it will be noted that the colloidal particles migrate to the pole of the battery having a charge opposite to that of the colloidal particles. Thus, if the colloidal particles migrate to the negative pole, as indicated in Fig. 8, they must be positively charged. This migration of charged particles in an electric field is called **electrophoresis.**

Isoelectric Point. It is possible by proper means, such as changing the hydrogen-ion concentration of the colloidal solution, to remove the charge on the colloidal particles. When this is done, the particles no longer migrate in an electric field, and we say that the colloidal solution is at its **isoelectric point.** At this point substances in colloidal dispersion are most easily precipitated.

Origin of Charge. The next problem which confronts us is to explain the origin of the charge on a colloidal particle. It is not possible to state definitely how a given charge originates, but it is possible to advance very plausible theories. We will illustrate by means of two common colloidal systems. A gold sol may be easily prepared in the laboratory by the treatment of a gold chloride solution with sodium citrate and hydrogen peroxide. The negative charges on the gold particles may be explained by assuming that the gold particles adsorb some negative ion present in the solution, possibly the citrate or chloride ion.

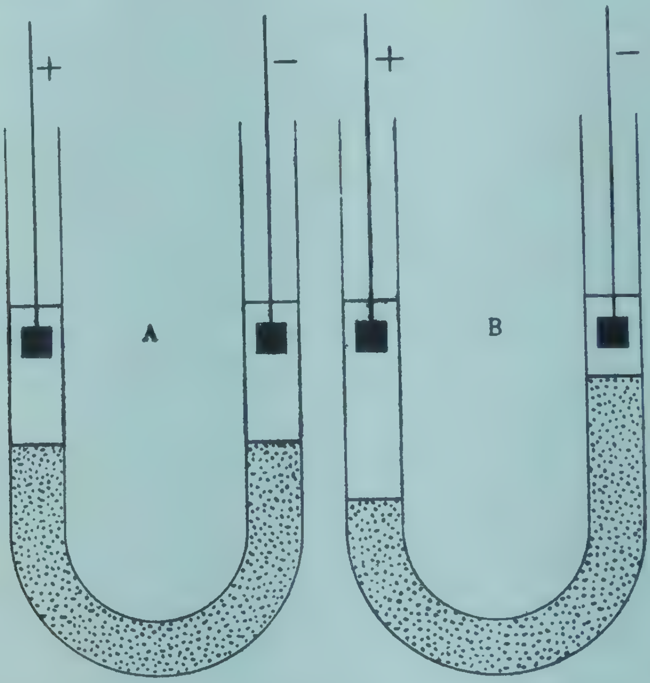


FIG. 8. Apparatus for demonstrating electrophoresis; *A*, before, and *B*, after, electrophoresis.

A good example of a positive colloidal system is ferric hydroxide sol, which is prepared by adding $FeCl_3$ solution to boiling water. The $FeCl_3$ is hydrolyzed thus:

$$FeCl_3 + 3H_2O \rightarrow Fe(OH)_3 + 3HCl$$

The particles of $Fe(OH)_3$ may still contain some $FeCl_3$, forming a complex represented by the formula $[Fe(OH)_3]_xFeCl_3$. The $FeCl_3$ in this complex may ionize, forming Fe^{+++} and $3Cl^-$, the 3 Cl^- going into solution and the Fe^{+++} remaining with the ferric hydroxide particle and giving it its positive charge.

$$[Fe(OH)_3]_xFeCl_3 \rightarrow [Fe(OH)_3]_xFe^{+++} + 3Cl^-$$

Precipitation of Colloids. In a preceding section it was pointed out that there are two types of colloidal systems, namely, suspensoids and emulsoids. Suspensoids, being much more readily precipitated, are considerably less stable than emulsoids. In a suspensoid system the particles have no affinity for the dispersion medium and remain dispersed because of the **electric charges** present. In an emulsoid system the particles have an affinity for the dispersion medium, and in a hydrophilic system may be considered as having a layer of water around them which aids in stabilizing the sol. Thus there are two factors favoring stability in an emulsoid system, namely, **electric charge** and **hydration.** The relationship between suspensoids and emulsoids and precipitated particles is diagrammatically represented in Fig. 9.

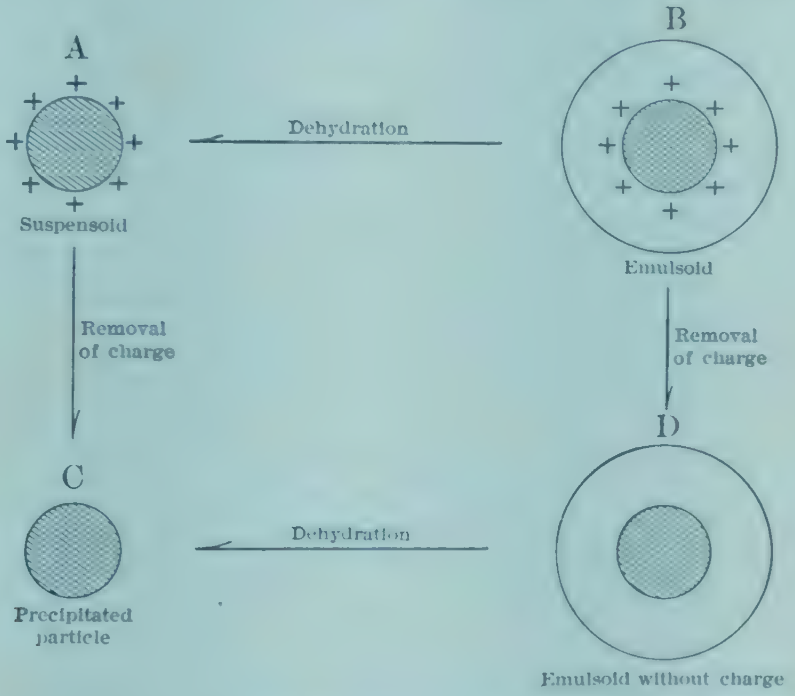


FIG. 9. Relationship between suspensoids and emulsoids and precipitated particles.

In Fig. 9, *A* represents a suspensoid particle with its electric charges, *B* an emulsoid particle with its electric charges and its layer of water, *D* an emulsoid particle with no charge but with a layer of water, and *C* a precipitated colloidal particle with no charge or layer of water.

To precipitate *A*, all that is necessary is to remove the charge; to precipitate *B*, it is necessary to remove the charge and also the water; to precipitate *D* it is necessary to remove only the water.

The precipitation of suspensoids may be accomplished by mixing two colloidal sols having opposite charges. This precipitation is often demonstrated in the laboratory by adding to a negative gold sol some positive ferric hydroxide sol. If the two are mixed in the proper proportions, a precipitation will occur. This method of precipitation is spoken of as **mutual precipitation** because both sols are precipitated.

Another method of precipitating suspensoids is by the addition of salt solutions. In this case the ion having the opposite charge to that on the colloidal particle is the precipitating ion. Ions vary greatly in their ability to precipitate sols. The main factor which determines this ability is the valence of the ion. From this statement it might be thought that Al^{+++} would be three times as potent a precipitating agent for a negative sol as Na^+. Experiment shows, however, that it is from 200 to 1600 times as potent. When Al^{+++} strikes a negative colloidal particle, it delivers three positive charges simultaneously, whereas to get the same effect three Na^+ must strike the particle at the same time. In order to have three charges strike a particle simultaneously, the concentration of Na^+ must be very high in comparison to that of Al^{+++}.

From this discussion the conclusion might be drawn that the precipitated suspensoid particle is still of colloidal size. This is undoubtedly not true. When the charge is removed from a suspensoid particle, the force which tends to keep the particles apart no longer exists, and therefore many particles unite to form larger particles, thus aiding precipitation.

In order to precipitate an emulsoid it is necessary to remove the water from the colloidal particle as well as the charge. The presence of either charge or hydration is sufficient to stabilize an emulsoid colloid. It is possible to remove the charge on an emulsoid or remove the water and still have a stable sol. However, if both charge and water are removed sufficiently, precipitation occurs. A good example of an emulsoid colloid is an albumin sol. Such a sol may be precipitated by saturation with ammonium sulfate. The first addition of ammonium sulfate probably neutralizes the charge on the albumin particles, but, because of the hydrophilic nature of albumin, the sol remains stable. On the further addition of ammonium sulfate, water is removed from the colloidal particles until at complete saturation the albumin precipitates. The precipitation of colloids by the use of concentrated salt solutions is often spoken of as **salting out.**

Besides the chemical methods just described for separating colloidal particles from their dispersion medium, two physical methods are frequently used. One method is called **ultracentrifugation** and the other **ultrafiltration.**

An ultracentrifuge is an apparatus that rotates liquids at extremely high speeds. By means of the ultracentrifuge it is possible to separate colloidal particles from their dispersion medium by centrifugal force which throws the denser material farthest from the center of rotation. Particles in true solution cannot be separated from their solvent by this means.

Ultrafiltration differs from filtration in that a membrane is used in place of a filter paper and pressure is applied to the solution being filtered. Since colloidal particles will not pass through most membranes, it is possible to force the dispersion medium through an appropriate membrane, leaving the colloidal particles inside the membrane. Some membranes are porous enough to allow small colloidal particles to pass through them. Such membranes may be used to separate colloids of different sizes and to estimate the size of colloidal particles.

Protective Colloids. Emulsoids, as has been stated, are much more stable than suspensoids. If a small amount of an emulsoid is added to a suspensoid, the suspensoid becomes much more stable. The emulsoid forms a protective coating on the surface of the suspensoid particles, giving them much of the stability of the emulsoid. An emulsoid used in this manner is spoken of as a **protective colloid.**

Zsigmondy devised a method for measuring the protective ability of an emulsoid colloid. He determined what he called the **gold number.** This is the number of milligrams of protective colloid which, in 10 ml. of especially prepared red gold sol, will just fail to prevent a change from red to blue when 1 ml. of 10 per cent NaCl solution is added.

The gold number has been used quite extensively in medicine in the diagnosis of certain forms of insanity. Normal spinal fluid has a very definite gold number. In some kinds of insanity the gold number of the spinal fluid is altered to a marked degree.

Structure of Gels. If a sufficient amount of gelatin is dissolved in hot water, a gelatin sol is obtained that on cooling changes to a gel. What is the difference in structure between a sol and a gel which gives rise to such a marked difference in physical properties? It is believed that in the sol state the gelatin particles are separated from each other and are therefore free to move about, thus giving the sol the appearance of a liquid. Many theories have been advanced to explain the rigid properties of a gel. At the present time the most generally accepted theory concerning gel structure is that in gelation the colloidal particles arrange themselves in the form of fibrils, which assume what is called a "brush heap" structure. A gel, then, is a mass of these fibrils with a dilute sol enmeshed between them.

Coagulation. On being heated, a gelatin gel will again form a sol. A gelation which can pass from a gel to a sol and then back to a gel again is said to be reversible. Many sols form gels which cannot be readily reconverted to the sol again. Such a gelation is often spoken of as a **coagulation.** A good example of coagulation occurs on heating egg white. Coagulated egg white cannot be readily brought back into the sol form. In this connection it may be mentioned that Mona Spiegel Adolf has been able to reconvert especially purified, heat-coagulated, serum albumin to the sol form, which she claims is identical with the original sol.

Syneresis. On standing, gels often contract, squeezing out a dilute sol. This phenomenon is called **syneresis.** Syneresis is perhaps best exemplified by the separation of serum from clotted blood on standing. In the preparation of junket and custard pies the clotted casein often contracts on standing, with the separation of a watery liquid. Syneresis is of great importance in physiology. It probably plays a part in such vital phenomena as the formation of secretions and the functioning of the muscle fibers.

Imbibition. Many gels, when placed in water, swell and take on water. This phenomenon is called **imbibition.** In many ways imbibition may be looked upon as the opposite of syneresis. Hydrogen-ion concentration and salts have a great influence on the amount of water that certain gels will imbibe.

The Colloidal State as an Aid to Chemical Reactions. In the discussion of surface tension it was pointed out that substances which lower surface tension tend to concentrate in the surface film. This phenomenon was called adsorption. In a colloidal system the surface area becomes enormously increased, since the interface between each colloidal particle and the dispersion medium is a surface. Adsorption, then, becomes an extremely important factor in dealing with colloidal systems. Undoubtedly the colloidal nature of protoplasm has much to do with the remarkable chemical changes which take place in it.

There are three ways in which the colloidal nature of protoplasm may aid in bringing about chemical reactions. First, by adsorption on the surface films the reacting molecules are brought into close contact. This increases the concentration of the reacting substances, which most certainly hastens their interaction. Second, by concentration at the surface, surface tension is reduced and consequently energy is released. This energy may be converted into chemical energy, which may be used for the energy requirements involved in the chemical transformations which are taking place. Third, the energy, instead of being released, may be used to produce more surface, thus giving a greater area for adsorption.

The Donnan Equilibrium

If a vessel is divided into two compartments by a membrane permeable to water and simple ions like Na^+ and Cl^-, and the compartments are filled with solutions of NaCl of unequal concentrations, ions will diffuse through the membrane until the concentrations of Na^+ and Cl^- are the same in both compartments and the products of the concentrations of the two ions are the same on both sides of the membrane.

Donnan has pointed out that the situation is altered when an ion on one side of the membrane is unable to diffuse through the membrane. In such a case the concentrations of the diffusible ions vary on each side of the membrane but the product of the concentrations of these ions is the same on both sides of the membrane when equilibrium is established.

Let us suppose that we have a condition represented by the following diagram, where the vertical line represents the membrane:

$$\begin{array}{c|c} K^+ & K^+ \\ R^- & Cl^- \end{array}$$

In this diagram all the ions are free to pass through the membrane except R^-. According to Donnan's theory, K^+ and Cl^- will pass through the membrane until the concentration of K^+ × concentration of Cl^- is the same on both sides of the membrane. If the concentrations of the various ions were equal to begin with, then K^+ and Cl^- would pass from right to left until the conditions just mentioned were met, which would mean that the number of K^+ on the left-hand side of the membrane would be greater than the number on the right-hand side. Also the number of Cl^- on the left-hand side of the membrane will be less than on the right-hand side. Furthermore, the total number of ions on the left will be greater than on the right because of R^- ions which are unable to diffuse through the membrane. Also for every R^- there must be a K^+ in order to maintain electrical neutrality. If the two solutions had equal osmotic pressures to start with, and if the volumes of the two solutions were kept constant, after equilibrium was reached the solution on the left-hand side would have a higher osmotic pressure than that on the right-hand side, since osmotic pressure depends upon the number of ions or molecules present in a given volume of solution.

In order to clarify what has just been said the following diagram is presented.

$$\begin{array}{r|l} 90\text{K}^+ & 90\text{K}^+ \\ 90\text{R}^- & 90\text{Cl}^- \end{array}$$

A

$$\begin{array}{r|l} 120\text{K}^+ & 60\text{K}^+ \\ 90\text{R}^- & \\ 30\text{Cl}^- & 60\text{Cl}^- \end{array}$$

B

In these diagrams the vertical lines represent membranes, through which all ions can diffuse except R^-. Let *A* represent the original conditions with 90 K^+ and 90 R^- on the left and with 90 K^+ and 90 Cl^- on the right. It should be noted that the total number of ions is the same on each side of the membrane and that therefore the osmotic pressure of the two solutions should be the same.

Let *B* represent conditions after equilibrium has been established. It will be noted that 30 K^+ and 30 Cl^- have diffused through the membrane from right to left. On the left there are now 120 K^+, 90 R^-, and 30 Cl^-, and the product of $\text{K}^+ \times \text{Cl}^-$ is 120×30 or 3600. On the right-hand side of the membrane there remain 60 K^+ and 60 Cl^-, and the product of $\text{K}^+ \times \text{Cl}^-$ is 60×60 or 3600. Thus the products of the concentrations of K^+ and Cl^- are equal on both sides of the membrane, which satisfies Donnan's theory. It will be noted that there are 240 ions on the left side of the membrane and 120 on the right. Thus, if the volumes of the two solutions remain constant the osmotic pressure of the solution on the left should be twice that on the right. Also the number of K^+ on the left is now twice that on the right and the number of Cl^- on the left is half that on the right. Thus we have two solutions, separated by a membrane, in which the concentrations of the diffusible ions are decidedly varied.

Donnan's theory is of great importance in biology because it explains how the concentrations of diffusible ions may differ inside and outside of cells. It explains the passage of ions in and out of cells and gives us an explanation of many of the acts of secretion and absorption which are continually going on in the various organs of the body. It also explains differences in *p*H which often exist inside and outside a cell.

Optical Activity

A very important physical phenomenon frequently encountered in biochemistry is optical activity. This was discovered by Biot in 1815. By optical activity is meant the ability of a compound to rotate a plane of polarized light. Optical activity is shown by crystals of some inorganic compounds, such as quartz, certain organic liquids, such as turpentine, and by solutions of many organic compounds, such as carbohydrates. To understand optical activity one must first become familiar with the subject of polarized light.

Polarized Light. A ray of ordinary light vibrates in all directions at right angles to the direction in which the ray is traveling. An approaching ray of light may be imagined to appear like the spokes of a wheel, with the spokes representing the vibrations radiating in all directions. (See Fig. 10.) If a ray of ordinary light is passed through a crystal of calcium carbonate, known as Iceland spar, the ray is split into two diverging rays. These rays, instead of vibrating in all directions, now vibrate in single planes at right angles to each other and are said to be **plane polarized.** If light is passed through a **Nicol prism** (see Fig. 11), which is made of two pieces of Iceland spar cemented together by Canada balsam, it is possible to deflect one of these rays so that only one ray of polarized light passes through the Nicol prism. Solutions of many substances with which we will deal in biochemistry have the power of rotating such a ray of plane-polarized light. If the plane of polarized light is rotated to the right, the substance is said to be **dextrorotatory.** If the ray is rotated to the left, the substance is said to be **levorotatory.** Since the degree of rotation is characteristic of the substance and is proportional to the concentration of the substance in solution, we have a very valuable method available for the quantitative estimation of such a substance.

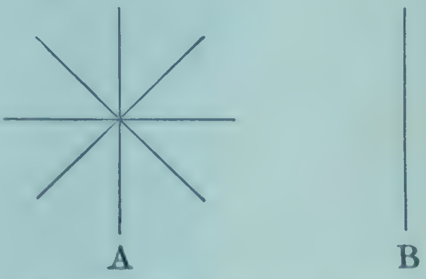


FIG. 10. The difference between ordinary and plane-polarized light. *A* is ordinary light, vibrating in all planes. *B* is plane-polarized light as it emerges from a Nicol prism, vibrating in only one plane.

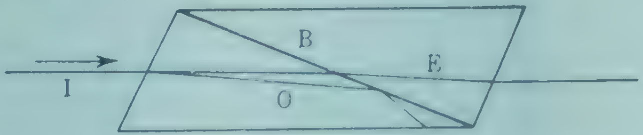


FIG. 11. Diagram of a Nicol prism. Two triangular pieces of Iceland spar are cemented together with Canada balsam, *B*. The incident ray of light, *I*, on entering the prism is doubly refracted. The ordinary ray, *O*, upon striking *B*, is reflected out of the prism. The extraordinary ray, *E*, travels through the prism and emerges as a plane-polarized beam.

Polariscope. In order to determine the rotatory ability of a substance a special instrument has been developed which is called a polariscope. (See Figs. 12 and 13.) In its simplest form a polariscope consists of two prisms, made of Iceland spar, separated from each other by a dark tube. The prism nearer the source of light is in a fixed position and is known as the **polarizing prism.** The other, nearer the eye of the observer, is movable and is called the **analyzing prism.** If the two

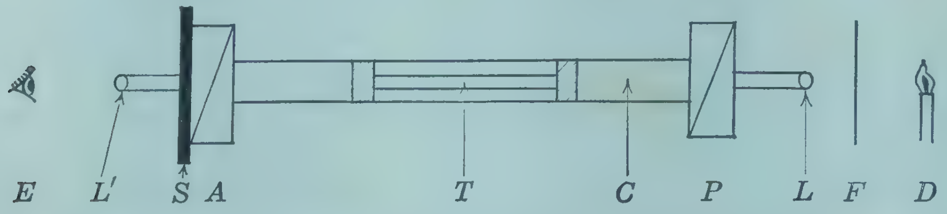


FIG. 12. The essential features of a polariscope. *D* is a source of yellow light; *F* is a light filter allowing only yellow light to pass through; *L* is a lens for focusing the light on the stationary polarizing Nicol prism, *P*; *C* is a dark chamber for holding the polariscope tube, *T*, which contains the solution to be analyzed; *A* is the analyzing Nicol prism, which may be rotated; *S* is a scale attached to *A* for measuring the amount of rotation; *L′* is a lens for observing the light passing through *A*; *E* is the eye of the observer.

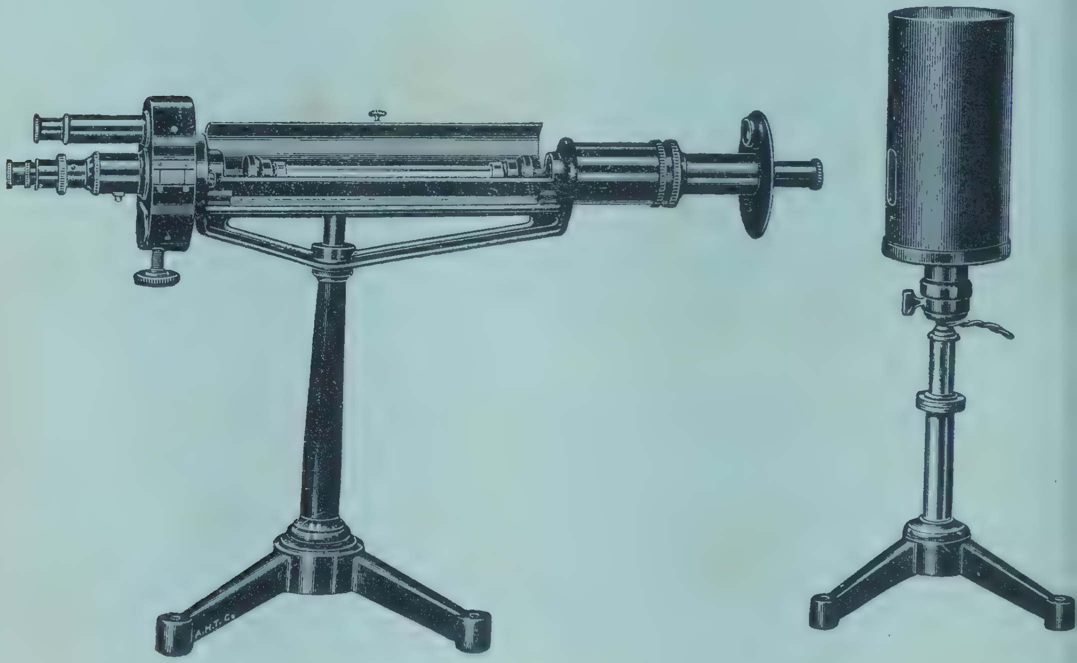


FIG. 13. A Schmidt and Haensch saccharimeter; a polariscope designed for sugar work.

prisms are arranged so that their optical axes are in the same plane, light will pass through both prisms. If the optical axis of the analyzing prism is at right angles to that of the polarizing prism, no light will pass through the analyzing prism. In a polariscope the analyzing prism is arranged so that it may be rotated. A scale indicates the number of degrees through which it is rotated. The **zero point** on the scale may be at the point where the optical axes of the two prisms are at right angles to each other and no light will pass through the instrument. The solution to be analyzed is placed in a tube of known length and introduced into the dark tube connecting the two prisms. Light passing through the polarizing prism is plane polarized. The plane of polarized light in passing through the optically active substance is rotated either to the right or to the left. On striking the analyzing prism some of the light passes through, because the plane of light is not at right angles to the optical axis of the analyzing prism. By rotating the analyzing prism, a point is reached where no light passes through it. The optical axis of the analyzing prism is then at right angles to the plane of polarized light. By noting the scale, the angle through which the analyzing prism was rotated can be determined. This is equal to the angle through which the plane of polarized light was rotated by the solution in the tube. The direction of rotation, either to the right or the left, can also be determined.

Since different wavelengths of light are rotated to different degrees by optically active substances, it is necessary to use **monochromatic light** in polariscopic work. Yellow light, which is known to the physicist as the D line of the spectrum, is ordinarily used. As temperature also influences the rotatory power of substances, it is customary to make polariscopic readings at 20°C.

Specific Rotation. In order to standardize polariscopic work, the term **specific rotation** has been introduced. In formulas, specific rotation is represented by $[\alpha]$. For example, $[\alpha]_D^{20°}$ means specific rotation at 20°C., where the D line of the spectrum is used. The specific rotation of a substance is the rotation in angular degrees of a solution of 1 gram in 1 ml. concentration when read in a tube 1 decimeter long. Since we cannot always work with solutions of this concentration, and since the rotation is proportional to the concentration, we can determine the specific rotation of a substance by examining a solution of any known concentration and substituting in the following formula:

$$[\alpha]_D^{20°} = \frac{\alpha}{lC}$$

where α is the observed rotation, l is the length of the tube in decimeters, and C is the concentration, that is, the grams of substance in 1 ml.

Optical Isomerism. Many compounds of biological interest have the power of rotating the plane of polarized light. Perhaps the best examples are to be found among the carbohydrates. Many sugars which have the same empirical formula are quite different in their power to rotate polarized light. Compounds which have the same empirical formula but which differ in their behavior toward polarized light are said to be **optical** or **stereo isomers.**

A very simple compound which displays this type of isomerism is a sugar containing three carbon atoms and called **glycerose.** There are three varieties of glycerose. One variety, when in solution, rotates the plane of polarized light to the right and is called **dextroglycerose.** Another rotates the plane of polarized light to the left and is called **levoglycerose.** A third variety is optically inactive and is called **racemic glycerose.** (See p. 42.)

Four different varieties of tartaric acid are known. One rotates the plane of polarized light to the right and is called **dextrotartaric acid.** Another rotates the plane of polarized light to the left and is called **levotartaric acid.** Besides these there are two varieties of optically inactive tartaric acid, one called **racemic tartaric acid** and the other **mesotartaric acid.** (See p. 42.)

Racemic Mixtures. The reason why racemic tartaric acid is optically inactive was explained by Pasteur. He noted that, when a solution of the sodium ammonium salt of racemic tartaric acid was allowed to crystallize, two kinds of crystals were obtained. Although they were similar in general appearance, they actually were quite different as one kind was the mirror image of the other. He separated the two kinds of crystals by means of tweezers, and on dissolving one variety in water he obtained a solution which rotated the plane of polarized light to the right, whereas he found that a solution of the other variety rotated the plane of polarized light to the left.

In other words, he showed that racemic tartaric acid was a mixture of 50 per cent dextro- and 50 per cent levotartaric acid. The optical inactivity of racemic glycerose can be explained on the same basis. It is possible to separate racemic glycerose into its two optically active components.

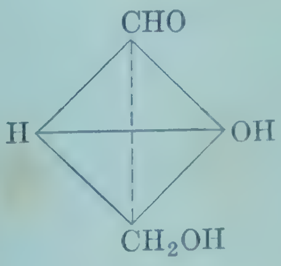
Resolution of Racemic Mixtures. The separation or **resolution** of racemic mixtures into their optically active constituents is important not only from the theoretical but also from the practical standpoint. Frequently either the dextro or the levo variety of an optically active compound is desired when only the racemic mixture is available. Obviously the method used by Pasteur, known as the **mechanical method,** for the resolution of a racemic mixture is tedious and impractical.

The method usually employed is known as the **chemical method.** It involves preparing a derivative in which the dextro and levo varieties have different solubilities. If racemic tartaric acid is treated with an alkaloid, such as strychnine, brucine, or cinchonine, the alkaloid will react with the two forms of tartaric acid to form a mixture of the dextrotartaric acid and the levotartaric acid derivatives of the alkaloid. These differ in their solubilities and may be separated by fractional crystallization.

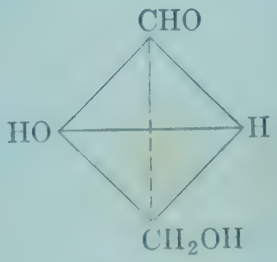
A third method, known as the **biological method**, may be employed. Many microorganisms, when grown on a racemic mixture of an optically active compound, will destroy one variety and leave the other unchanged. The unchanged variety can then be separated from the solution in a pure form. For example, a species of the mold *Penicillium* will attack only dextrotartaric acid when grown on a solution of racemic tartaric acid. This mold can then be used for preparing levotartaric acid from racemic tartaric acid. Of course this method is of value only when the desired variety is not destroyed by the microorganism.

Explanation of Optical Activity. The fundamental explanation of optical activity was propounded by Van't Hoff and Le Bel. These men worked independently, but both advanced the same theory about the same time. They noted that every carbon compound which showed optical activity contained at least one carbon atom which had attached to it four different elements or groups. Such a carbon atom was said to be **asymmetric.** They advanced the theory that optical activity is due to the presence of an asymmetric carbon atom in the molecule.

Optical isomerism can best be visualized by representing the asymmetric carbon atom as a tetrahedron, in which the four corners are the four valences of carbon. The formulas for dextro- and levoglycerose would then be represented as follows:

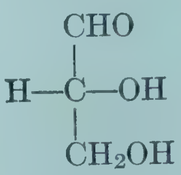


Dextroglycerose



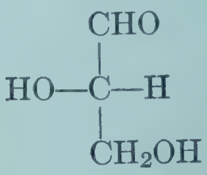
Levoglycerose

It will be noted that these two formulas differ in that H and OH are interchanged. That this difference is important is recognized if an attempt is made to superimpose one formula on the other. It is readily seen that these two tetrahedrons cannot be superimposed on each other and still have the positions of all the groups attached to the asymmetric carbon correspond. It is also evident that one structure is the mirror image of the other. These different arrangements of the groups tied to an asymmetric carbon atom account for the existence of two varieties of a compound containing such a carbon atom. Since it would obviously be difficult to write formulas in which carbon is represented by a tetrahedron, it is customary to use ordinary graphic formulas and to indicate the differences in optically active substances by the position of the groups to the right or to the left of the asymmetric carbon atom. Thus the formulas for the two varieties of glycerose would be:



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H—C—OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

Dextroglycerose

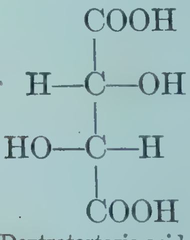


$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HO—C—H} \\ | \\ \mathrm{CH_2OH} \end{array}$$

Levoglycerose

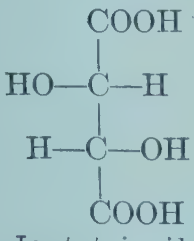
Racemic glycerose
(a 50–50% mixture of dextro- and levoglycerose)

Tartaric acid has two asymmetric carbon atoms, both of which are asymmetric in the same way; that is, the four different groups tied to each asymmetric carbon atom are the same. If both of the asymmetric carbon atoms have the dextro arrangement, we have a molecule which is strongly dextrorotatory. This is dextrotartaric acid. If both the asymmetric carbon atoms are levorotatory, we have levotartaric acid. Racemic tartaric acid is a 50 per cent mixture of each of these varieties. It is said to be optically inactive on account of **external compensation.** If one of the asymmetric carbon atoms of tartaric acid is dextrorotatory and the other levorotatory, then one asymmetric carbon atom neutralizes the rotatory power of the other, and we have optical inactivity due to **internal compensation.** This internally compensated tartaric acid is mesotartaric acid.



$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{H—C—OH} \\ | \\ \mathrm{HO—C—H} \\ | \\ \mathrm{COOH} \end{array}$$

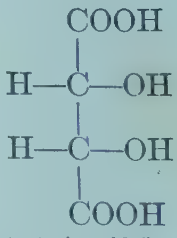
Dextrotartaric acid



$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{HO—C—H} \\ | \\ \mathrm{H—C—OH} \\ | \\ \mathrm{COOH} \end{array}$$

Levotartaric acid

Racemic tartaric acid (inactive)



$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{H—C—OH} \\ | \\ \mathrm{H—C—OH} \\ | \\ \mathrm{COOH} \end{array}$$

Mesotartaric acid (inactive)

In these formulas it will be noted that the two central carbon atoms are asymmetric. At first sight it appears that the two asymmetric carbon atoms in dextrotartaric acid, for example, do not have the same arrangement of groups attached to them because the OH groups are on opposite sides of the molecule. However, if the formula is rotated clockwise 180°, it can be seen that the arrangement of groups is the same as it was before rotation. Thus both carbon atoms have the same arrangement of groups tied to them; and, if this arrangement causes a rotation of polarized light to the right, the compound should be doubly active in its dextrorotatory properties. The same explanation applies to levotartaric acid. In mesotartaric acid it will be noted that the top asymmetric carbon atom is dextro and the bottom one levo. Thus the rotatory power of one asymmetric carbon atom neutralizes that of the other, and mesotartaric acid has no rotatory power.

We have used for our discussion of optical activity glycerose and tartaric acid, both of which are rather simple compounds. It should be noted at this point that most of the organic compounds of biological importance are much more complicated, and most of those in which optical activity exists have many asymmetric carbon atoms. Hence the possibilities for optical isomerism are greatly increased. In a rather simple compound like glucose, assuming a free aldehyde structure, there are four asymmetric carbon atoms, and the number of possible optical isomers is sixteen. In more complicated compounds, such as the proteins, the possibilities for optical isomerism are much greater.

Before leaving the subject of optical isomerism a word should be said about the modern method of naming optically active isomers. In place of the words dextro and levo the letters D and L are used, but D and L no longer refer to the direction of optical rotation but rather to whether or not an isomer is structurally related to dextro- or levoglyceric aldehyde. To indicate the direction of rotation (+) or (−) signs are used following the D or L. A (+) sign indicates rotation to the right and a (−) sign rotation to the left. For example, in the above discussion of the tartaric acids, levotartaric acid is actually the D variety and would be referred to as D(−)-tartaric acid, the D indicating that it is related structurally to dextroglyceric aldehyde and the (−) to the fact that it is levorotatory. Dextrotartaric acid is L(+)-tartaric acid. The inactive forms of compounds containing asymmetric carbon atoms, namely, the racemic and meso varieties, are often referred to as DL and M, respectively.

Isotopes

When the atomic theory was originally proposed by John Dalton it was assumed that all atoms of a given element were identical and had the same atomic weight. As time went on it became apparent that many elements exist in more than one form, each form having a different atomic weight but the same chemical properties. These different forms of the same element are called isotopes. **Isotopes** may be defined as elements that have identical chemical properties but differ in their atomic weights.

Structure of the Atom. An atom is made up of a **nucleus** surrounded by negatively charged units called **electrons.** The nucleus contains positively charged units called **protons** and electrically neutral units called **neutrons.** Protons and neutrons have about the same mass and are about 1850 times as heavy as electrons. Thus the atomic weight of an element depends largely on the number of protons and neutrons present. The chemical properties of an element, on the other hand, depend largely on the number of electrons present. Isotopes of an element have the same number of negatively charged electrons, and since the atom is normally electrically neutral, the number of protons must also be the same. Isotopes differ in the number of their neutrons. The number of neutrons present affects the atomic weight but not the chemical properties of the element.

An ordinary hydrogen atom is made up of a nucleus composed of one proton and one planetary electron and has an atomic weight of 1. An isotope of hydrogen, called heavy hydrogen or **deuterium,** is made up of a nucleus composed of one proton and one neutron and one planetary electron, and has an atomic weight of 2. Thus the difference between ordinary hydrogen and deuterium is that deuterium contains a neutron and hydrogen does not. A third variety of hydrogen called **tritium** contains one proton, two neutrons, and one planetary electron and has an atomic weight of 3. In referring to elements which occur as isotopes it is customary to indicate the isotope by a superscript, denoting its atomic weight, following the symbol of the element. Thus ordinary hydrogen is written H^1, deuterium H^2, and tritium H^3. Of the common elements found in biological materials, namely carbon, nitrogen, oxygen, phosphorus, and sulfur, several isotopes of each are known.

Types of Isotopes. *Stable Isotopes.* There are two types of isotopes, stable and radioactive. Stable isotopes are those most commonly found in nature and may be obtained by such methods as fractional distillation of compounds containing the isotopes of the element desired. For example, water containing a mixture of H^1O and H^2_2O could be separated by this means.

The amount of deuterium in an organic compound may be determined by oxidizing the compound, which converts the hydrogen and deuterium present into water. The water is collected and its specific gravity determined. From the specific gravity the proportion of H^1_2O and H^2_2O present can be determined.

For determining stable isotopes of other elements an instrument called a **mass spectrometer** is used. The element to be determined is converted into a gas such as carbon dioxide or nitrogen. The molecules of gas are then ionized and passed through the instrument which is a complicated device for separating ionized molecules of different masses by passing them through a magnetic field.

Radioactive Isotopes. Radioactive isotopes of biologically important elements are prepared by bombarding nonradioactive atoms with subatomic particles by means of such a device as a cyclotron. Radioactive isotopes may be determined by the amount of radiation they give on decomposition. The measurement is made with a Geiger counter. When an ionized particle enters the instrument a current of electricity flows which activates a counting device producing a click. The number of clicks per unit of time is proportional to the concentration of the radioactive element in the material being examined.

Use of Isotopes in Biochemistry. The chemical changes which occur in living matter are very complicated. When we eat food it is digested and absorbed into the blood which carries it to the tissues. Here complicated chemical reactions occur until finally the food molecules are broken down into rather simple molecules which are eliminated from the body through the kidneys and lungs. Until recently little was known concerning the details of the chemical reactions going on in the tissues. It is now possible to synthesize certain simple food molecules so that they contain isotopic elements such as hydrogen, oxygen, carbon, and nitrogen. These isotopes act as labels on food molecules making it possible to trace them through the various stages of their metabolism. The living organism does not distinguish between food molecules containing isotopes and those that do not. Thus by using isotopes much knowledge has been gained regarding the details of what happens to foods during metabolism.

REVIEW QUESTIONS

1. Name three biologically important properties of water and state the importance of each.

2. State Avogadro's law.

3. Define osmotic pressure, semipermeable membrane, osmosis, dialysis, hypertonic solution, hypotonic solution, isotonic solution, physiological salt solution, plasmolysis, and plasmoptysis.

4. If 50 grams of a compound which does not ionize is dissolved in 1 liter of water and the osmotic pressure is found to be 10 atmospheres, what is the mole-

ular weight of the compound? What are the freezing and the boiling points of the solution?

5. If 10 grams of a compound which does not ionize is dissolved in 1 liter of water and the freezing point is $-0.31°C.$, what is the molecular weight of the compound? What are the osmotic pressure and the boiling point of the solution?

6. How may the freezing point of milk be used to tell the amount of water added to milk?

7. The molecular weight of a compound which does not ionize is 200. If 50 grams is dissolved in 1 liter of water, what will the osmotic pressure and the boiling and the freezing points of the solution be?

8. If the osmotic pressure of a solution is 10 atmospheres, what are the freezing and the boiling points of the solution?

9. What is meant by colligative properties of solutions?

10. What is meant by surface tension? Give a theory explaining it.

11. Define adsorption and negative adsorption.

12. Distinguish between the quantity and the intensity factors in acidity.

13. What is a normal solution of an acid? Of a base? What is a standard solution? What is a normality factor? What is meant by titratable acidity?

14. If a solution has a *p*H of 6, what is the *p*OH? Is the solution acidic, basic, or neutral?

15. Name two general methods for determining *p*H.

16. Name three electrometric methods for determining *p*H.

17. Describe a colorimetric and an electrometric method for determining *p*H.

18. What is meant by a buffer solution?

19. Define an indicator as applied to acids and bases.

20. What indicator would you use in titrating a weak acid against a strong base? A weak base against a strong acid?

21. How did Thomas Graham classify substances on the basis of diffusibility?

22. Compare in outline form true solutions, colloidal solutions, and suspensions.

23. Describe an ultramicroscope.

24. What is meant by Brownian movement and the Tyndall phenomenon?

25. Define dispersed phase, dispersion medium, suspensoid, emulsoid, hydrophobic, hydrophilic, sol, and gel.

26. Define electrophoresis and isoelectric point.

27. Explain how the charge on a colloidal particle may originate.

28. Discuss the precipitation of colloids.

29. What is meant by a protective colloid? What is the gold number?

30. Discuss the structure of gels.

31. What is meant by coagulation, syneresis, and imbibition?

32. Explain how the colloidal state may be an aid to chemical reactions taking place in protoplasm.

33. Discuss Donnan's theory of membrane equilibrium.

34. Define optical activity, polarized light, Nicol prism, dextrorotatory, monochromatic light.

35. Draw a diagram of a polariscope, indicating the important features.

36. Define specific rotation.

37. Name and account for three varieties of glycerose and four varieties of tartaric acid.

38. What is an asymmetric carbon atom? A racemic mixture? External compensation? Internal compensation?

39. How may a racemic mixture be separated into its optically active components?

40. What do D, L, DL, M, (+), and (−) mean when placed before the name of an optically active compound?

41. Define isotopes.

42. What are the differences in structure between hydrogen, deuterium, and tritium atoms?

43. Name two types of isotopes and characterize each.

44. How are stable isotopes identified?

45. How are radioactive isotopes prepared and identified?

46. How are isotopes used in biological research?

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3

Carbohydrates

From the biochemical viewpoint three of the most important classes of organic compounds are the carbohydrates, lipids, and proteins. In this chapter we will consider the carbohydrates. The carbohydrates are considered first, because from the chemical standpoint they are the simplest, and because they form a part of some of the more complex lipids and proteins. The carbohydrates are the most abundant constituent of many foods and are the source of a large part of the heat and mechanical energy of the body. Although the carbohydrates are usually thought of as a source of heat and energy, it should not be overlooked that they also occur as a part of protoplasm and must therefore be essential for the building of body tissue.

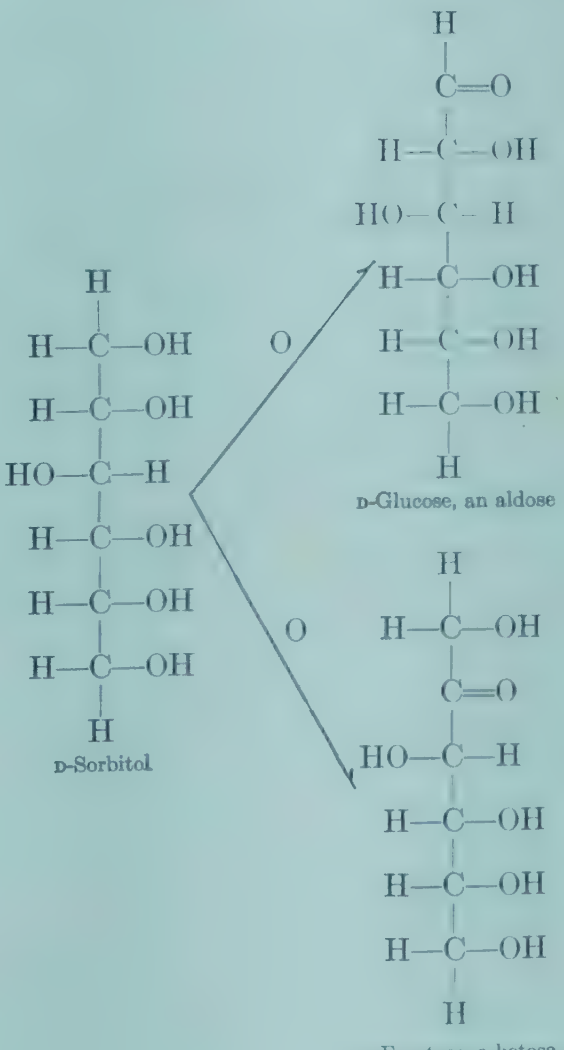
The most abundant carbohydrate found in nature is cellulose, which is the main constituent of woody tissue, the material which gives plants their structure. Although cellulose cannot be utilized as a food by man, it is found in many of his foods. The starches, also occurring abundantly in nature, are the main source of carbohydrate in the diet. Simpler sugars, such as glucose, sucrose, and lactose, are also important sources of carbohydrate in nutrition.

Perhaps the most important generalizations that can be made concerning the composition of carbohydrates are that they are composed of carbon, hydrogen, and oxygen, and that the hydrogen and oxygen are usually in the proportion of two to one, just as in water. Because of this fact they were considered, in the early days, to be hydrates of carbon and for this reason were called carbohydrates. The name is misleading, however, because water does not exist as such in a carbohydrate. It is simply a coincidence that in carbohydrates hydrogen and oxygen have this relationship.

It should be noted at this point that not all organic compounds containing hydrogen and oxygen in the proportion found in water are carbohydrates. For example, acetic acid (CH_3COOH) is not a carbohydrate. Also, some carbohydrates, such as rhamnose ($C_6H_{12}O_5$), do not contain the usual proportions of hydrogen to oxygen.

The simplest carbohydrates, sometimes called **monosaccharides** or **simple sugars,** are derivatives of polyhydric alcohols. If one alcohol group in a polyhydric alcohol is oxidized to either an aldehyde or a ketone, the result is a simple carbohydrate. Thus a simple carbohydrate may be defined as the first oxidation product of a polyhydric alcohol in which either an end alcohol group is oxidized to form an aldehyde or some other alcohol group (usually the second) is oxidized to form a ketone. Thus we have two kinds of simple carbohydrates or sugars: aldehyde alcohols, called **aldoses,** and ketone alcohols, called **ketoses.**

The most common simple sugars with which we have to deal are **D-glucose** and **D-fructose.** These are oxidation products of the polyhydric alcohol **D-sorbitol,** and the relationships are indicated in the accompanying formulas.



D-Sorbitol

D-Glucose, an aldose

D-Fructose, a ketose

Classification of Important Carbohydrates

- I. Monosaccharides or simple sugars.
    - 1. Pentoses, $C_5H_{10}O_5$.
        - *a.* Aldoses.
            - L-Arabinose.
            - D-Xylose.
            - D-Ribose.
    - 2. Hexoses, $C_6H_{12}O_6$.
        - *a.* Aldoses.
            - D-Glucose.
            - D-Galactose.
            - D-Mannose.
        - *b.* Ketoses.
            - D-Fructose.
- II. Disaccharides, $C_{12}H_{22}O_{11}$.
    - 1. Those which reduce Fehling's solution.
        - Maltose (D-glucose + D-glucose).
        - Cellobiose (D-glucose + D-glucose).
        - Lactose (D-glucose + D-galactose).
        - Melibiose (D-glucose + D-galactose).
    - 2. Those which do not reduce Fehling's solution.
        - Sucrose (D-glucose + D-fructose).
- III. Trisaccharides, $C_{18}H_{32}O_{16}$.
    - Raffinose (D-fructose + D-glucose + D-galactose).
- IV. Tetrasaccharides, $C_{24}H_{42}O_{21}$.
    - Stachyose (D-fructose + D-glucose + D-galactose + D-galactose).
- V. Polysaccharides.
    - 1. Pentosans, $(C_5H_8O_4)_x + H_2O$.
        - Araban (L-arabinose).
        - Xylan (D-xylose).
    - 2. Hexosans, $(C_6H_{10}O_5)_x + H_2O$.
        - *a.* Glucosans.
            - Starch.
            - Dextrin.
            - Glycogen.
            - Cellulose (normal).
        - *b.* Fructosans.
            - Inulin.
        - *c.* Mannosans.
            - Vegetable ivory.
    - 3. Mixed polysaccharides.
        - Gums.
        - Mucilages.
        - Hemicelluloses.
        - Compound celluloses.

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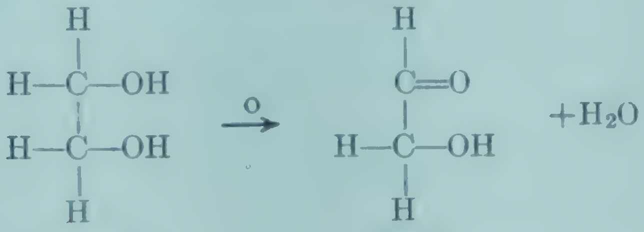
The simple carbohydrates are classified on the basis of the number of carbon atoms in the chain. That with two carbon atoms is called a **diose;** those with three, **trioses;** with four, **tetroses;** with five, **pentoses;** with six, **hexoses;** etc. With the exception of the diose, each of these groups is again divided into aldoses and ketoses, depending on whether it contains aldehyde or ketone groups. The di-, tri-, tetra-, and polysaccharides are sometimes called **compound sugars.** They are made up of monosaccharide units linked together by the splitting out of water.

Simple Sugars

Diose. The simple sugars are those which cannot be broken down by hydrolysis into simpler sugars. The simplest compound which corresponds to our definition of a carbohydrate is **glycollic aldehyde,** the first oxidation product of ethylene glycol.

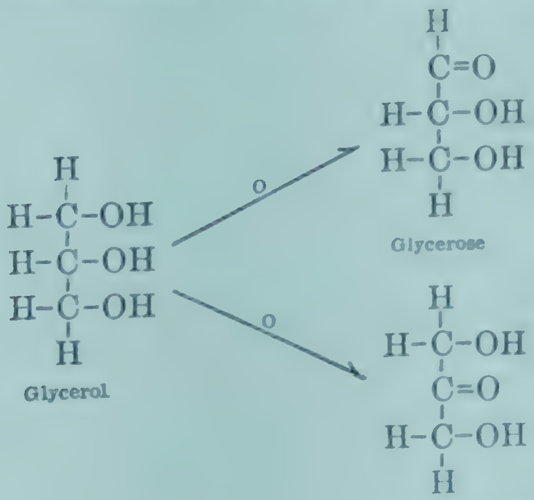


Ethylene glycol

Glycollic aldehyde

Glycollic aldehyde is a diose sugar of the aldose variety. Since both of the alcohol groups in ethylene glycol are primary, it is impossible to obtain a ketone on oxidation. An examination of the formula for glycollic aldehyde shows that it contains no asymmetric carbon atom, and therefore it exists in only one form; in other words, it has no optical isomer. There is, then, only one diose sugar: glycollic aldehyde.

Trioses. The trioses are oxidation products of the trihydric alcohol glycerol. In this case both an aldose and a ketose are possible. The

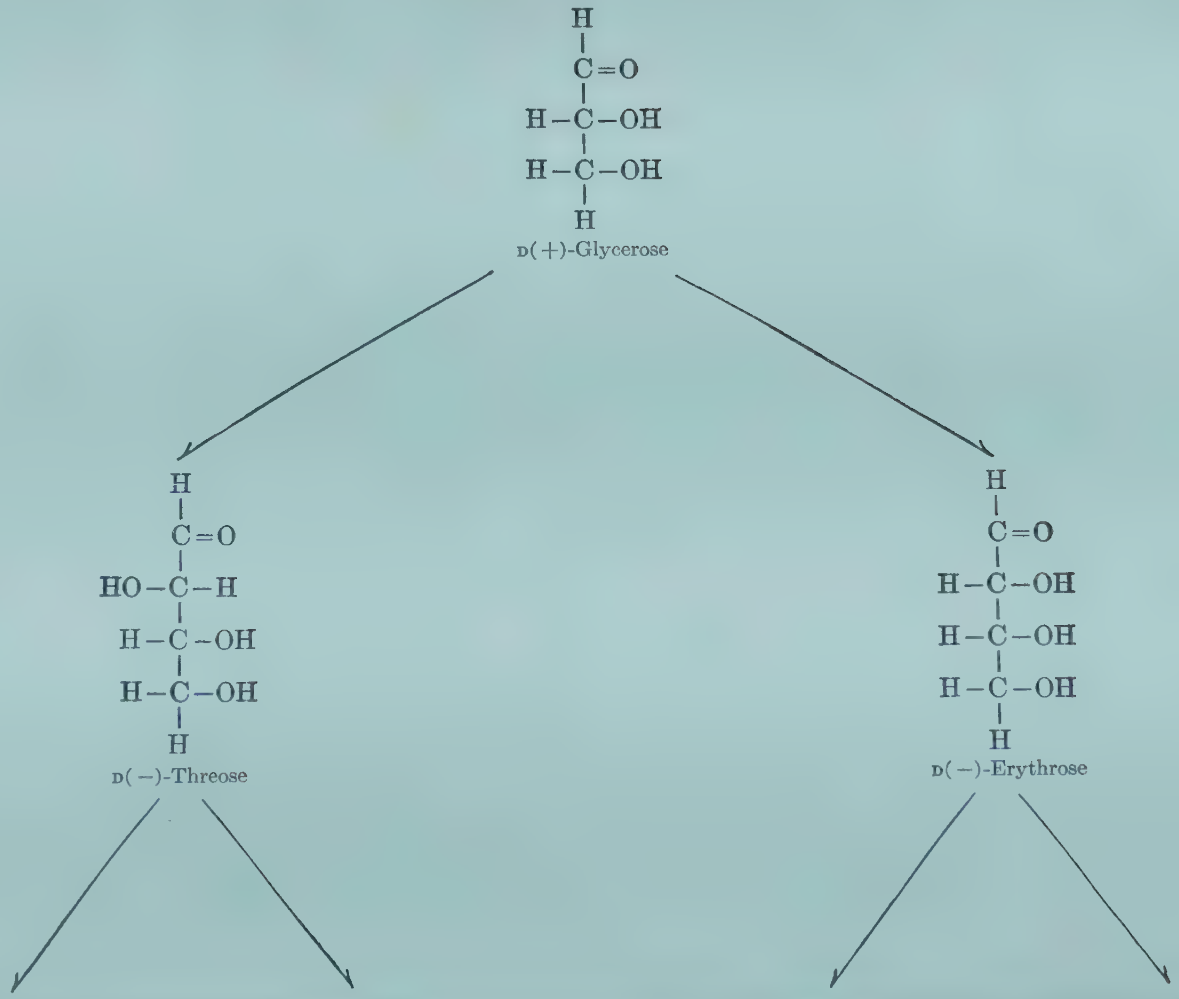


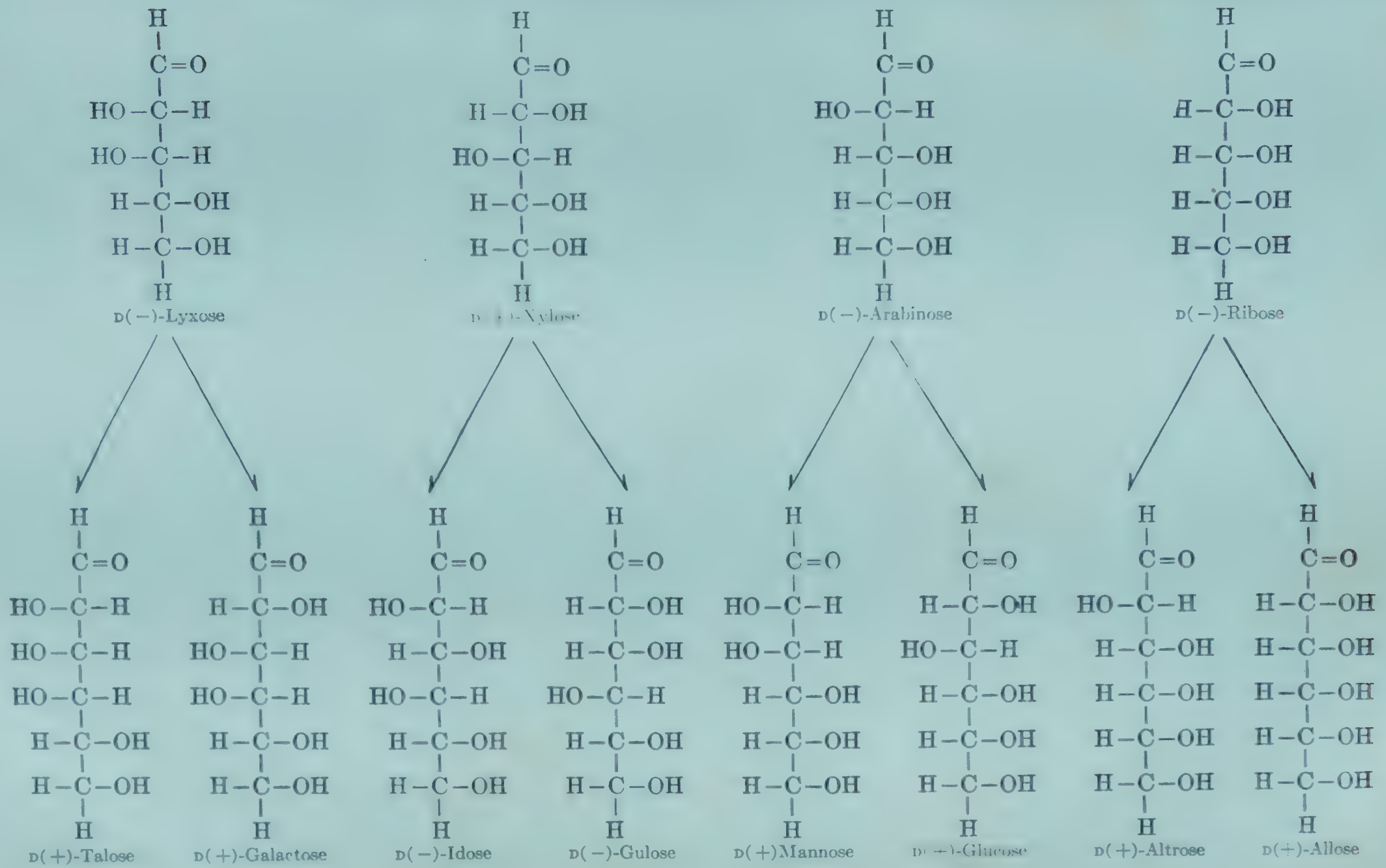
Glycerose

Glycerol

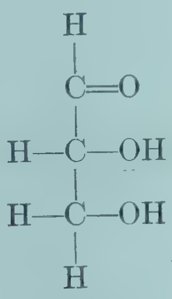
Dihydroxyacetone

THE D-ALDOSES

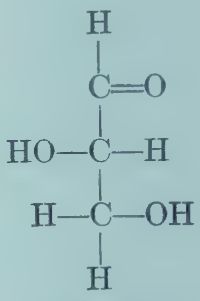

D(+)-Glycerose
D(−)-Threose
D(−)-Erythrose


D(−)-Lyxose
D(+)-Xylose
D(−)-Arabinose
D(−)-Ribose
D(+)-Talose
D(+)-Galactose
D(−)-Idose
D(−)-Gulose
D(+)Mannose
D(+)-Glucose
D(+)-Altrose
D(+)-Allose

aldose is called **glycerose,** and the ketose **dihydroxyacetone.** A glance at the formula for glycerose shows that the central carbon atom is asymmetric. Therefore glycerose exists in two forms, one of which rotates polarized light to the right (D-glycerose) and the other to the left (L-glycerose). Their formulas are as follows:



D-Glycerose



L-Glycerose

It should be noted that D-glycerose has the OH group on the carbon atom next to the primary alcohol group on the right, whereas L-glycerose has this OH group on the left. This difference is important, because all the longer-chained sugars are considered as having been derived from D- or L-glycerose, and it is possible to tell whether a sugar is D or L by observing the position of the OH groups on the carbon atom next to the primary alcohol group. The letter D- or L- before the name of a sugar does not necessarily mean that it rotates polarized light to the right or left, but rather that it is structurally related to D- or L-glycerose.

The derivation of the tetrose, pentose, and hexose sugars of the D variety from D-glycerose is indicated on pp. 52 and 53.

In the outline on pp. 52 and 53 it should be noted that each sugar is derived from the one above it by introducing an HCOH group next to the aldehyde group. The sign in parentheses indicates the direction of rotation of the sugar, (+) for dextro and (−) for levo.

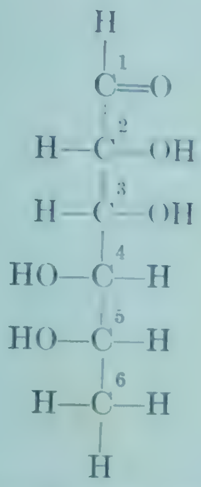
A similar series of sugars may be derived from L-glycerose, and therefore there are four tetroses, eight pentoses, and sixteen hexoses.

The number of optical isomers of a sugar is related to the number of asymmetric carbon atoms in the molecule and may be calculated by the use of the following formula:

$$(\text{Number of isomers} = 2^n)$$

in which n equals the number of asymmetric carbon atoms.

Desoxy Sugars. All the simple sugars mentioned so far have been aldehyde or ketone alcohols in which each carbon atom except the aldehyde or ketone carbon has had an OH group attached to it. All these sugars have corresponded to the general formula $C_nH_{2n}O_n$. There are compounds which are considered true sugars in which one or more of the OH groups have been replaced by H. These sugars, called desoxy sugars, are frequently encountered in biochemistry. A common desoxy sugar found in plants is **rhamnose**, which is 6-desoxy-L-mannose and has the following formula:



Rhamnose (6-desoxy-L-mannose)

The 6 refers to the carbon atom which has no OH group as the numbering proceeds from the aldehyde carbon. Rhamnose is sometimes spoken of as a methyl pentose.

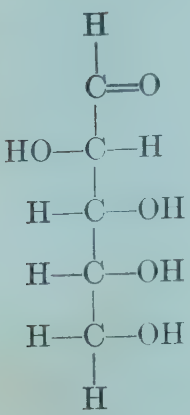
Since the pentoses and the hexoses, with their condensation products, are of most importance to the biochemist, we shall confine our discussion to these two groups.

Pentoses

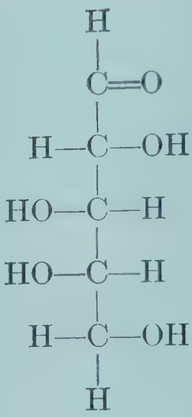
The pentoses are sugars with five carbon atoms. A glance at the formula for an aldopentose indicates that the molecule contains three asymmetric carbon atoms. This fact at once suggests great possibilities for optical isomerism. Applying the formula given above to an aldopentose, we find

$$(\text{Number of isomers} = 2^3 = 8)$$

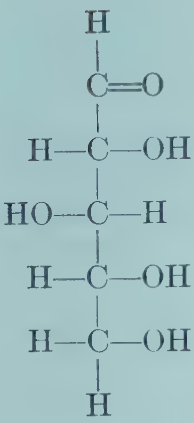
Of the eight aldopentoses only four are found in nature, namely, **D- and L-arabinose, D-xylose,** and **D-ribose.** D-Arabinose is found in certain glycosides, L-arabinose in gum arabic, D-xylose in wood and straw, and D-ribose in some nucleic acids. The following are the formulas for these pentoses:



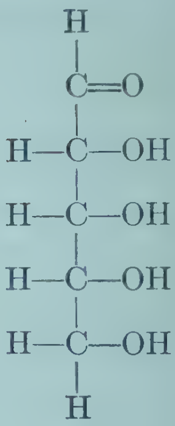
D-Arabinose



L-Arabinose



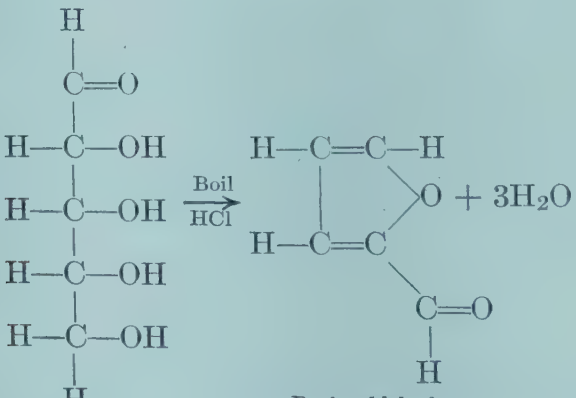
D-Xylose



D-Ribose

The pentoses occur quite commonly in plants in the form of polysaccharides called **pentosans.** They are not found to any great extent in animals. Under certain conditions, pentoses are present in human urine. Herbivorous animals can use them as food, but the extent to which man can utilize them is uncertain. As a food for human beings pentoses are unimportant.

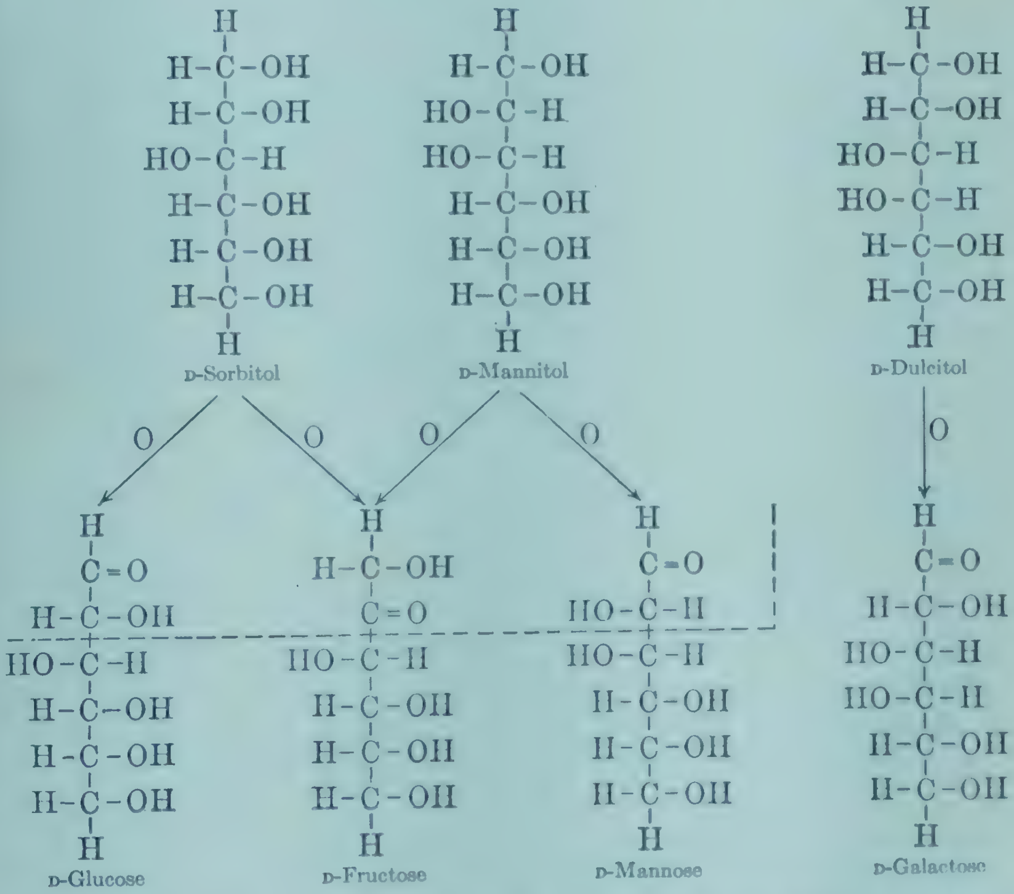
Pentoses may be distinguished from hexoses by the fact that common bread yeast will not ferment pentoses but will ferment hexoses. Often it is important to determine whether a sugar in solution is a pentose or a hexose. If such a solution is treated with bread yeast, and the sugar disappears after sufficient time has elapsed for fermentation to occur, the sugar must be a hexose. On the other hand, if the sugar remains after being exposed to yeast, it must be a pentose. Perhaps the most characteristic reaction of pentoses occurs on boiling with HCl, when pentoses decompose, forming **furfuraldehyde.**



Furfuraldehyde is volatile and may be detected in the distillate by the red color which it gives with aniline acetate. Furfuraldehyde also forms an insoluble compound with phloroglucinol. A method for the quantitative determination of pentoses is based upon this fact.

Hexoses

The most important carbohydrates from the standpoint of foods and nutrition are the hexoses and their condensation products. Only four of the hexose monosaccharides are important, namely, **D-glucose, D-mannose, D-galactose,** and **D-fructose.** These are oxidation products of the three polyhydric alcohols, **D-sorbitol, D-mannitol,** and **D-dulcitol.** Their relationships are evident from the following formulas:



These formulas show that D-glucose may be derived from D-sorbitol, D-fructose from either D-sorbitol or D-mannitol, D-mannose from D-mannitol, and D-galactose from D-dulcitol. The purpose of the dotted line between the second and third carbon atoms in the formulas for D-glucose, D-fructose and D-mannose is to emphasize the fact that in these three sugars the structures of the molecules differ only on the top two carbon atoms. If the fact that D-glucose has all its OH groups on the right-hand side of the molecule except on the third carbon atom is kept in mind, it is easy to recall the formula for each of the other sugars, for D-fructose differs from D-glucose in that it is a ketose; D-mannose differs on the second carbon atom, and D-galactose on the fourth carbon atom.

From the formulas it will be noticed that D-glucose has four asymmetric carbon atoms. Using the formula just discussed ($2^4 = 16$), we can see that there are sixteen possible isomers of D-glucose. Since D-fructose has three asymmetric carbon atoms, it is possible to have eight isomers of this sugar ($2^3 = 8$).

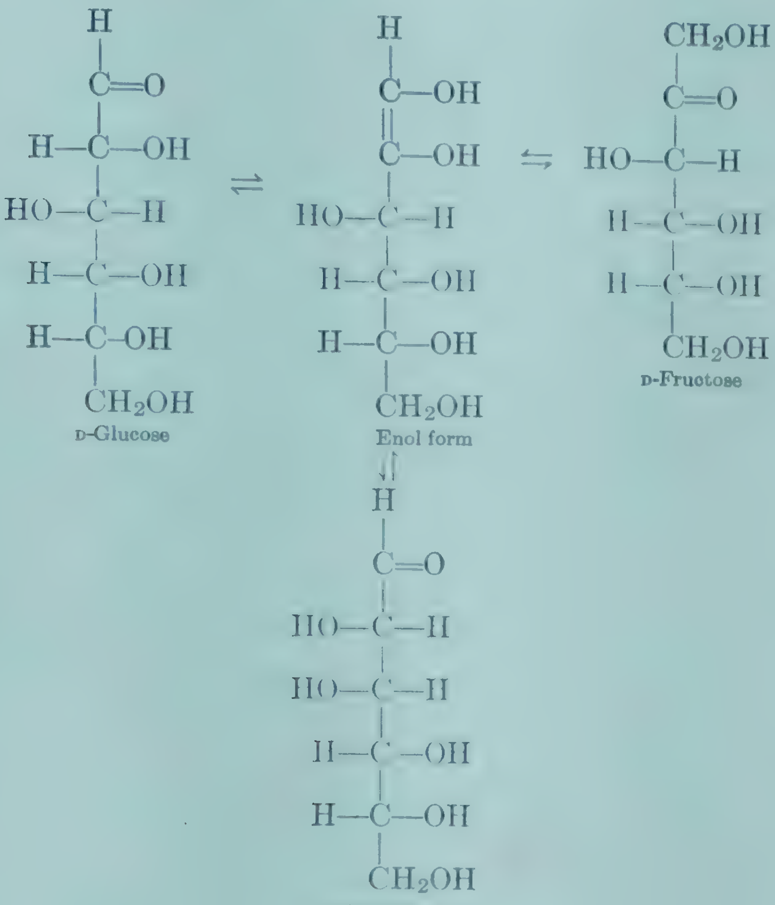
Reactions of Carbohydrates

Molisch's Test. The Molisch test is important because it is a test for carbohydrates in general. Any material containing carbohydrates will give a positive Molisch test. The test is performed by adding a few drops of an alcoholic solution of α-naphthol to the solution to be tested in a test tube. Concentrated H_2SO_4 is then poured down the side of the tube carefully so that it forms a layer at the bottom of the tube. A positive test is the formation of a violet color at the juncture of the two liquids. The reaction that occurs is the production of furfuraldehyde or related compounds by the action of the acid on the carbohydrate. The color is due to the action of these aldehydes on α-naphthol.

Moore's Test. The Action of Alkali on Carbohydrates. If a glucose solution to which has been added a solution of NaOH is boiled, a brown color will develop, and an odor of caramel can be detected arising from the solution. In the presence of strong alkali any carbohydrate having a free aldehyde or ketone group will be attacked and, on heating, will be broken down into reactive fragments. In the absence of oxygen these fragments unite to form caramel-like condensation products. If air or oxygen is bubbled through the solution during the heating, the brown color will not develop, for the reactive fragments will be completely oxidized. (In baking, if too much soda is used as a leavening agent, a tan product will be formed by the action of the alkali on the sugars present.)

In the presence of weak alkali the change in the sugar molecule is not so deep-seated. Rearrangement of the atoms in the molecule may take place, with the result that one sugar may change into another.

The interconversion of D-glucose, D-mannose, and D-fructose in the presence of weak alkali has been explained on the assumption that each of these sugars in the presence of alkali forms the same enol. This enol is unstable and tends to revert to the aldehyde or ketone form, giving a mixture of the three monosaccharides. Thus, starting with any one of the above sugars, one ends with a mixture of the three. This reaction is known as the **Lobry de Bruyn transformation.**



D-Glucose

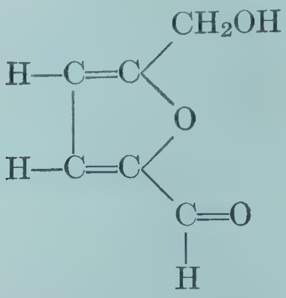
Enol form

D-Fructose

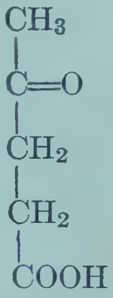
D-Mannose

Among the di- and polysaccharides we find that only those with a free carbonyl (C=O) group are attacked by alkali, and in those sugars the actions are similar to those discussed. A disaccharide like **sucrose,** which contains no free carbonyl group, is not acted on by even strong alkali. **Glycogen,** which is a polysaccharide, is not destroyed by boiling with 30 per cent NaOH solution.

Actions of Acids on Carbohydrates. In the last section we learned that most polysaccharides are very stable in the presence of alkalies. This is not true with acids. Di- and polysaccharides are easily hydrolyzed into their constituent monosaccharides when boiled with acid. We have already pointed out that pentoses are converted into furfuraldehyde when boiled with HCl. Hexoses under the same conditions give a variety of decomposition products, including hydroxymethyl furfuraldehyde, levulinic acid, formic acid, and carbon monoxide. Levulinic acid may be identified by the fact that it gives iodoform when treated with iodine in the presence of alkali.



Hydroxymethyl furfuraldehyde



CH_3—$C{=}O$—CH_2—CH_2—$COOH$

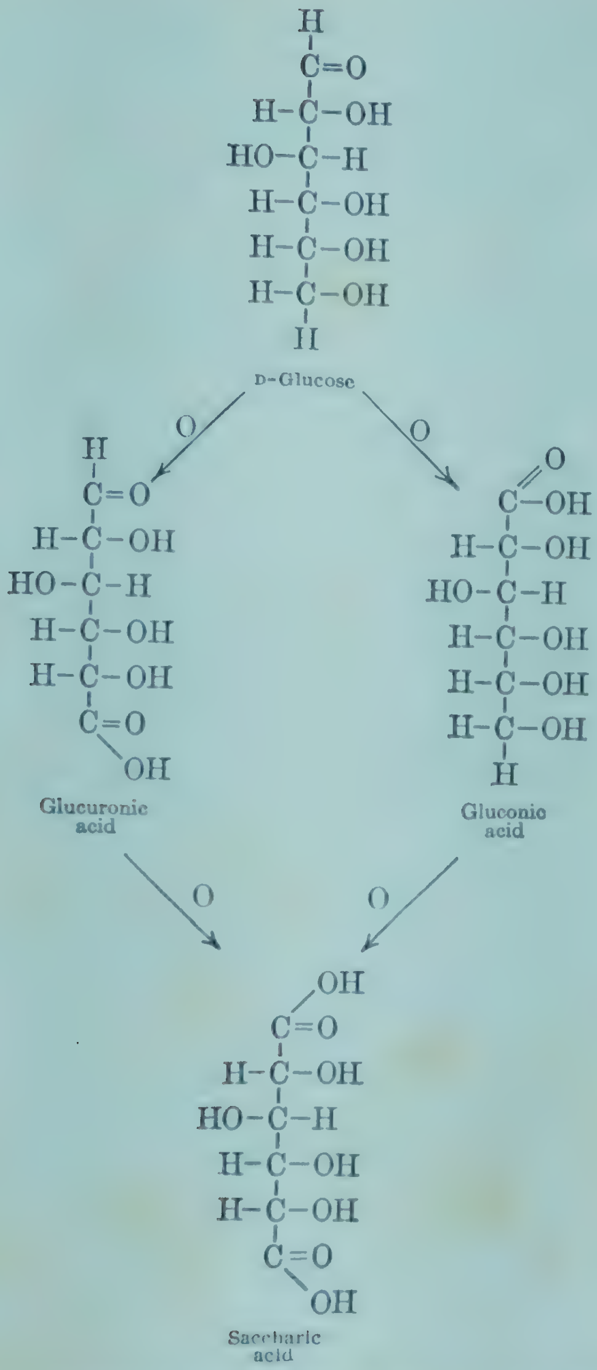
Levulinic acid

Oxidation of Carbohydrates. Since sugars contain alcohol and aldehyde or ketone groups, it is evident that they may be oxidized. In fact, the reducing property of sugars containing the free aldehyde or ketone group is one of their most important reactions from the chemist's standpoint, since this property is used in testing for sugar and determining the amount of it present in biological materials. If a simple sugar like glucose is oxidized, many products are formed. On complete oxidation, CO_2 and H_2O are formed. We will discuss only some of the oxidation products in which the molecule retains its six carbon atoms. Three such oxidation products of glucose are those represented by the formulas on p. 61.

The most important of these compounds physiologically is **glucuronic acid,** which is formed in the body and plays a vital role by uniting with many toxic substances to form inert paired compounds which are eliminated in the urine. This process is often spoken of as **protective synthesis,** because by this means the body protects itself against toxic substances. Some of the compounds made nontoxic by this process are chloral, benzene, nitrobenzene, aniline, phenol, turpentine, and camphor.

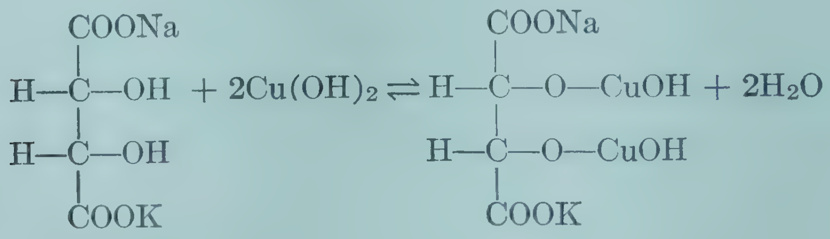
Other sugars oxidize in a manner similar to that of glucose, producing analogous products. It is common practice to speak of sugars with the primary alcohol group oxidized to carboxyl as **uronic acids.** Since this term is used frequently in biochemical literature, it is important to become familiar with it.

Fehling's Solution. Perhaps the most important reducing action of sugars with which the chemist is concerned is the reduction of copper salts to cuprous oxide by sugars which contain a free aldehyde or ketone group. The reaction is especially rapid in alkaline solution. It


D-Glucose
O
O
Glucuronic acid
Gluconic acid
O
O
Saccharic acid

will be recalled that strong alkali breaks up reducing sugars into reactive fragments. Since, in the presence of alkali, copper is precipitated as $Cu(OH)_2$, it is necessary to add something to the reagent to prevent this reaction. The substances used are generally salts of hydroxy acids. The OH groups tie up Cu, forming a complex soluble compound.

The best-known solution of this kind is Fehling's, which is made by adding to a solution of $CuSO_4$ a solution containing Rochelle salt and KOH or NaOH. The $CuSO_4$ is the oxidizing agent, the KOH makes the sugar more reactive, and the Rochelle salt prevents the precipitation of the Cu as $Cu(OH)_2$. The soluble Cu compound is probably formed thus:



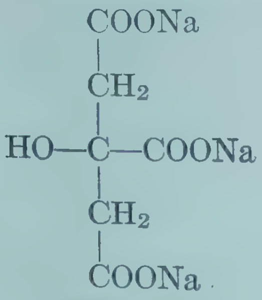
$$\begin{array}{l} \quad\text{COONa} \\ \quad\;| \\ \text{H—C—OH} \\ \quad\;| \\ \text{H—C—OH} \\ \quad\;| \\ \quad\text{COOK} \end{array} + 2Cu(OH)_2 \rightleftharpoons \begin{array}{l} \quad\text{COONa} \\ \quad\;| \\ \text{H—C—O—CuOH} \\ \quad\;| \\ \text{H—C—O—CuOH} \\ \quad\;| \\ \quad\text{COOK} \end{array} + 2H_2O$$

Rochelle salt
(sodium potassium tartrate)

The $Cu(OH)_2$ is in equilibrium with the complex tartrate. As fast as it is reduced, more is formed by a reversal of the reaction. The two constituents of Fehling's solution are not mixed until just before using, because the tartrate itself would tend to reduce the copper in time.

When a sugar solution containing a free aldehyde or ketone group is boiled with Fehling's solution, the characteristic result is a brick-red precipitate of Cu_2O. Sometimes the precipitate is yellow because of the formation of Cu(OH), which on long boiling should change to Cu_2O. A slight yellow precipitate often appears green because of the influence of the blue of Fehling's solution. If conditions are carefully controlled, Fehling's solution may be used for the quantitative determination of sugar by weighing the Cu_2O formed. Each milligram of Cu_2O indicates a definite weight of sugar.

Benedict's Solution. Another copper solution which is widely used in testing for sugars is Benedict's. It contains $CuSO_4$, Na_2CO_3, and sodium citrate. The main difference between it and Fehling's solution is that it contains Na_2CO_3 instead of KOH and is therefore not so strongly alkaline. It also contains sodium citrate in place of Rochelle



$$\begin{array}{l} \qquad\text{COONa} \\ \qquad\;| \\ \qquad\text{CH}_2 \\ \qquad\;| \\ \text{HO—C—COONa} \\ \qquad\;| \\ \qquad\text{CH}_2 \\ \qquad\;| \\ \qquad\text{COONa} \end{array}$$

Sodium citrate

salt as the salt of the hydroxy acid which prevents the precipitation of $Cu(OH)_2$. It has an advantage over Fehling's solution in that all the ingredients may be mixed together at once, forming a stable solution. Because of its weaker alkalinity it is more sensitive than Fehling's solution. The strong alkali of Fehling's solution tends to destroy traces of sugar, and hence small amounts of sugar may not be detected with Fehling's solution.

The solution just described is Benedict's qualitative reagent. There is also a Benedict's quantitative reagent, which has in it KSCN and $K_4Fe(CN)_6$ in addition to the ingredients in the qualitative reagent. When boiled with a solution of a reducing sugar, a white precipitate of CuSCN is formed instead of Cu_2O. The $K_4Fe(CN)_6$ prevents the precipitation of Cu_2O. The solution is made of such a strength that 25 ml. requires 50 mg. of glucose to reduce all the copper in it. For the test 25 ml. is measured into an evaporating dish and solid Na_2CO_3 is added. The sugar solution to be analyzed is then added from a burette to the boiling solution until all the copper is reduced, as indicated by the disappearance of the blue color. The volume of sugar solution added is then noted. In this volume there must have been 50 mg. of glucose. The solid Na_2CO_3 is added to maintain a saturated solution of Na_2CO_3 during the dilution process brought about by the addition of the sugar solution. Benedict's qualitative and quantitative solutions look alike, and it sometimes becomes necessary to distinguish between them. This can easily be done by adding to the solution in question some reducing sugar solution and boiling. The qualitative solution gives a brick-red precipitate, whereas the quantitative solution gives a white one.

Barfoed's Solution. Another common copper solution is Barfoed's reagent, which is unique in that it has an acidic instead of an alkaline reaction. It is made of cupric acetate to which have been added a few drops of acetic acid. As would be expected, sugar solutions do not reduce Barfoed's solution as readily as they do Fehling's and Benedict's solutions. In fact, disaccharides which reduce Fehling's solution rapidly reduce Barfoed's solution very slowly. Monosaccharides reduce it fairly rapidly forming Cu_2O. Because of these facts Barfoed's reagent is used to distinguish between mono- and disaccharides. When Barfoed's reagent is employed for this purpose, it must be kept in mind that continued boiling will effect a reduction even with disaccharides.

Nylander's Solution. Another solution which is used to test for reducing sugars is Nylander's reagent. It contains bismuth subnitrate, potassium hydroxide, and Rochelle salt. It is similar to Fehling's solution but contains bismuth subnitrate in place of copper sulfate. On boiling with a solution of a reducing sugar black metallic bismuth precipitates. Nylander's solution has certain advantages over copper solutions in testing for sugar in the urine as it is not reduced by uric acid and some other urinary constituents which reduce copper. The chemistry involved in the reduction of Nylander's reagent may be represented as follows:

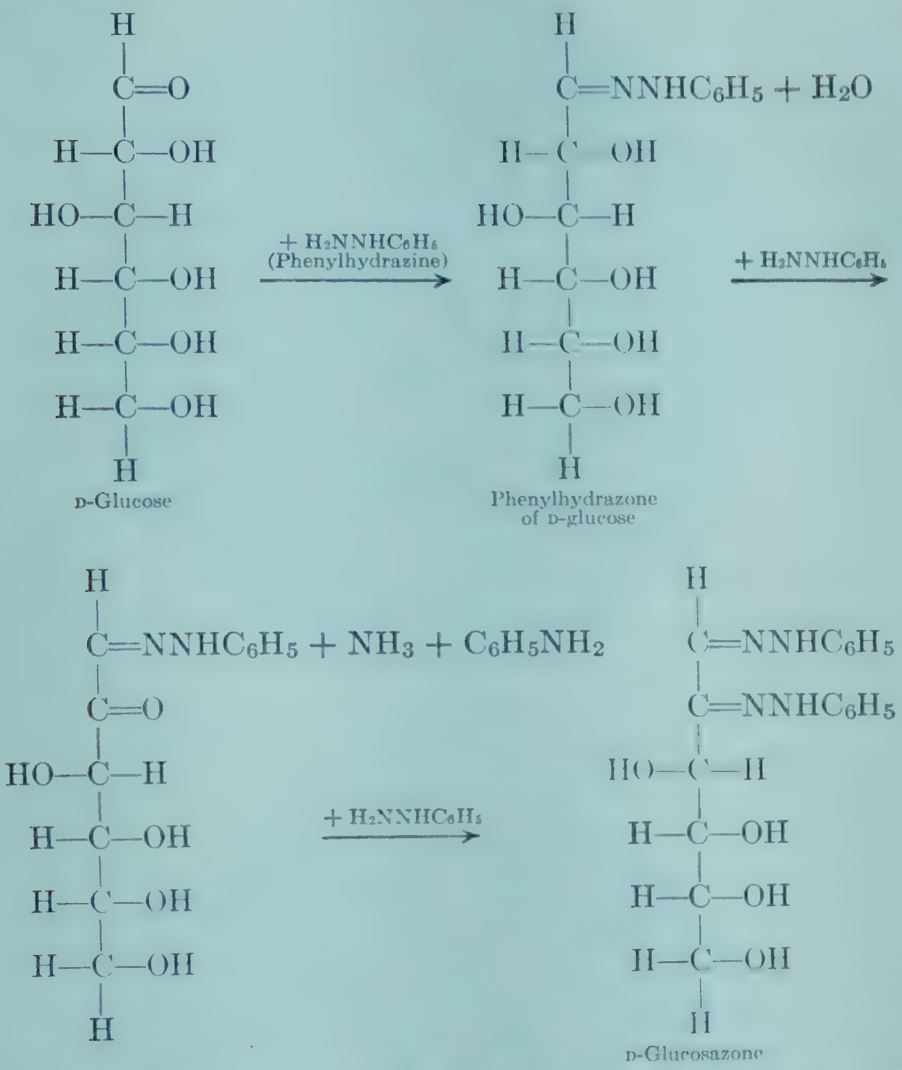
$$\mathrm{Bi(OH)_2NO_3 + KOH \rightarrow Bi(OH)_3 + KNO_3}$$
$$\mathrm{2Bi(OH)_3 \rightarrow 2Bi + 3H_2O + 3O}$$

Other Reagents Reduced by Sugars. Sugars will reduce many other compounds besides those of copper and bismuth. **Ammoniacal silver nitrate** solutions are reduced to metallic silver. **Potassium permanganate** solutions are reduced, as is indicated by the disappearance of their red color. In alkaline solution **picric acid** is reduced to picramic acid, which is mahogany red in color. This reaction is made the basis of a colorimetric method for the determination of sugar. In the presence of alkali, **methylene blue** is reduced to a colorless compound, and **phosphotungstic acid** to a blue compound, possibly an oxide of tungsten.

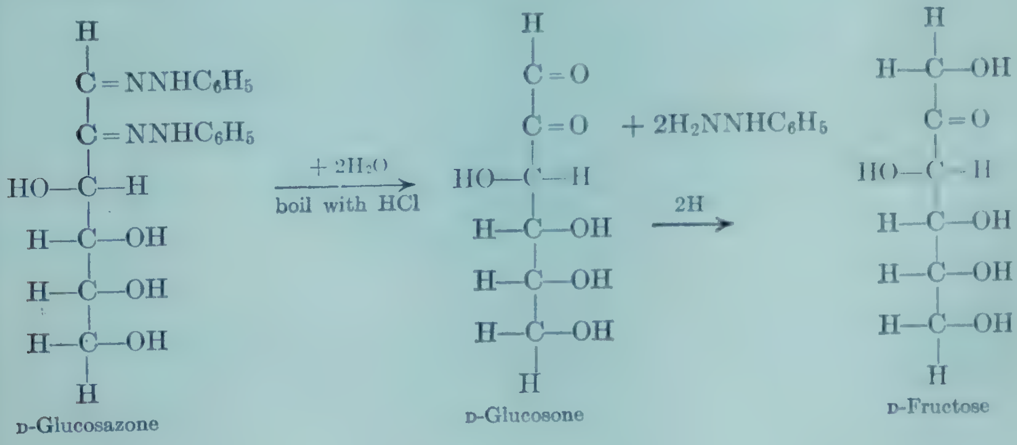
Reduction of Carbohydrates. In addition to being oxidized, carbohydrates may be reduced. If the aldehyde group of a simple sugar is reduced, the corresponding polyhydric alcohol is obtained. D-Glucose gives D-sorbitol; D-mannose, D-mannitol; D-fructose, both D-sorbitol and D-mannitol; and D-galactose, D-dulcitol. (See p. 57.) Mannitol is rather commonly found in nature and is probably formed by the reduction of fructose.

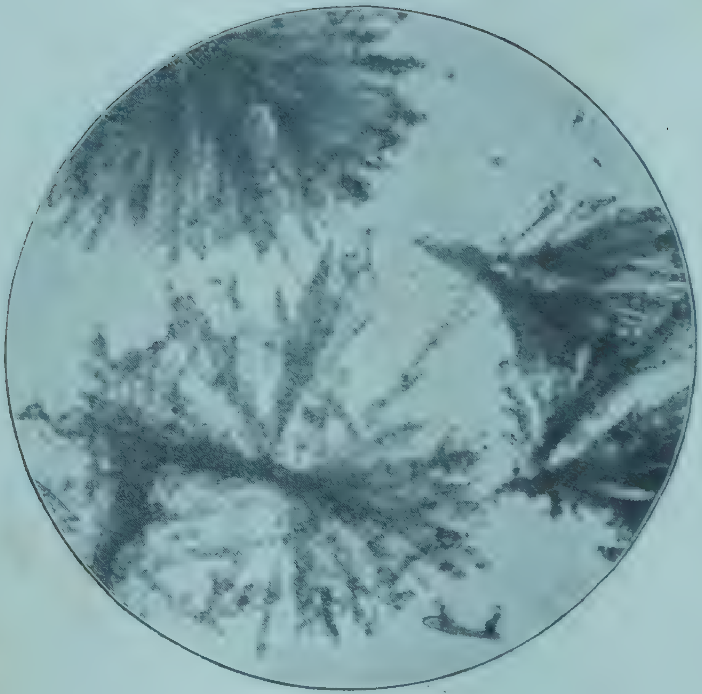
The formation of fats from sugar in the animal body must involve a reduction process, since fats contain much less oxygen than carbohydrates. That the excess of oxygen resulting from the conversion of carbohydrate to fat in the body can replace atmospheric oxygen obtained through respiration can be demonstrated experimentally. In an animal which is converting carbohydrate into fat the amount of oxygen consumed from the air is definitely less than when such conversion is not taking place. Oxidation in which the oxygen contained in food molecules is used is often spoken of as **anaerobic** or **intermolecular oxidation.**

Reactions of Carbohydrates with Phenylhydrazine. If a solution of a reducing sugar is heated with phenylhydrazine, a yellow precipitate is finally obtained. The precipitated compound is called an **osazone.** The reaction takes place in several steps thus:

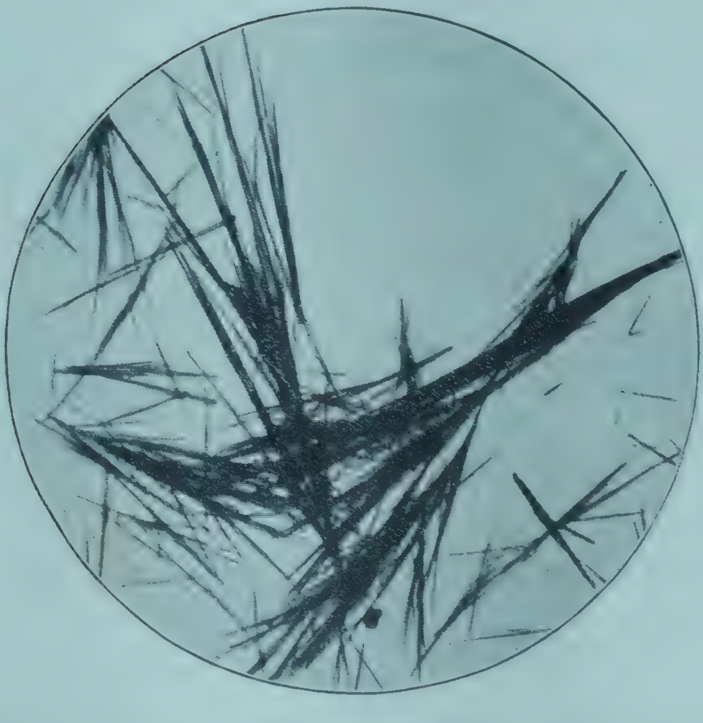

+ $H_2NNHC_6H_5$
(Phenylhydrazine)
+ H_2O
+ $H_2NNHC_6H_5$
+ NH_3 + $C_6H_5NH_2$
+ $H_2NNHC_6H_5$
D-Glucose
Phenylhydrazone of D-glucose
D-Glucosazone

If **D-glucosazone** is boiled with HCl, the two molecules of phenylhydrazine are split off, giving an **osone** which on reduction forms D-fructose thus:


+ $2H_2O$
boil with HCl
+ $2H_2NNHC_6H_5$
2H
D-Glucosazone
D-Glucosone
D-Fructose

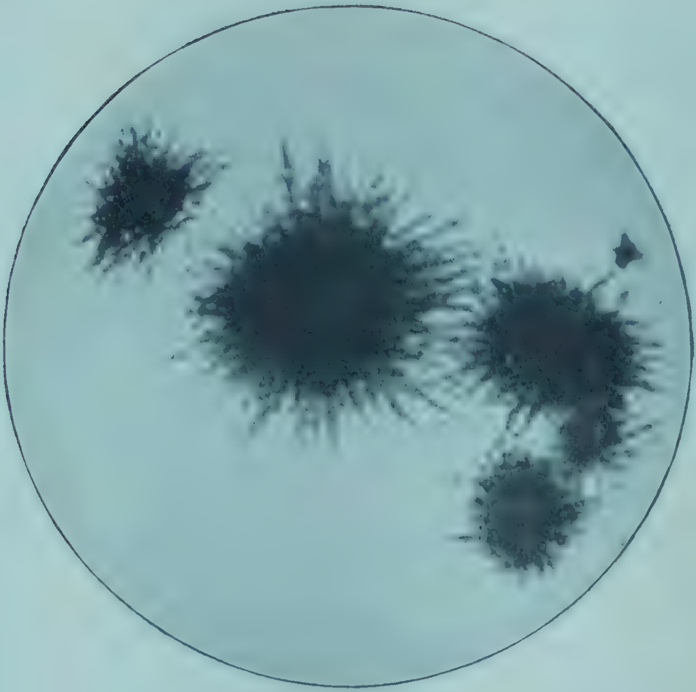


Galactosazone

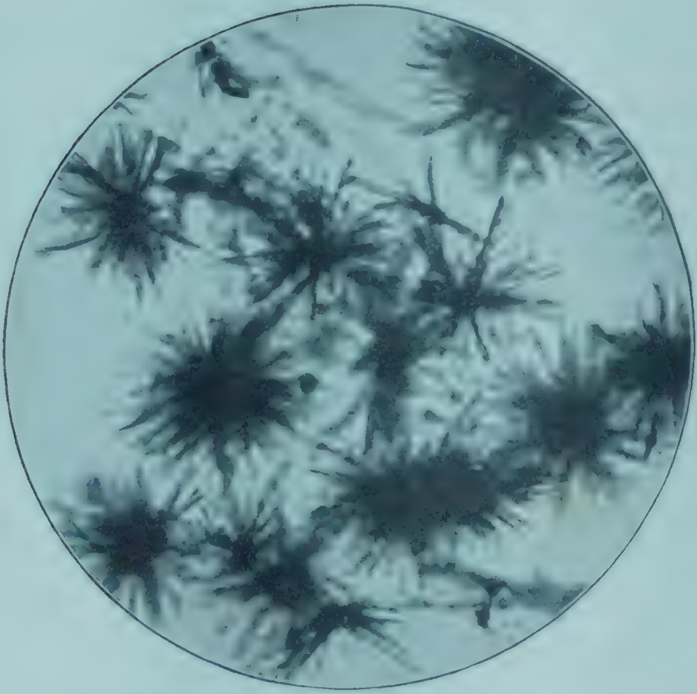


Glucosazone

Fig. 14. Osazones of common sugars. From *Biochemical Laboratory Methods* by Morrow and Sandstrom, John Wiley & Sons, by permission.



Lactosazone



Maltosazone

FIG. 14 (*continued*)

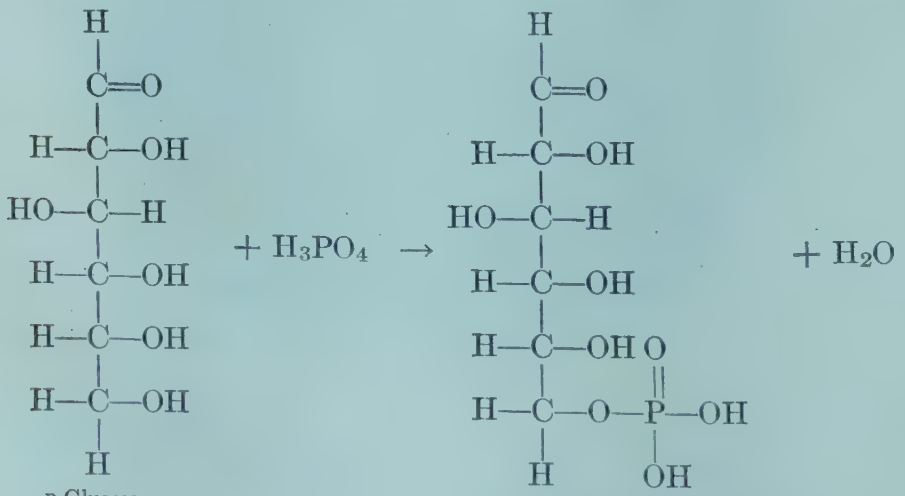
In osazone formation only the first two carbon atoms of the sugar are involved. Hence sugars which have the same arrangement on the last four carbon atoms will yield the same osazone. Since this is true of D-glucose, D-fructose, and D-mannose, all three of these sugars give the same osazone. The osazone of D-galactose is different, because the arrangement of groups on the fourth carbon atom is different. Since D-fructose may be made from D-glucose by the osazone reaction, it is evident that the two sugars have identical configurations on the last four carbon atoms.

Although D-mannose forms the same osazone as D-glucose, it should be noted that the hydrazone is different. The hydrazone of D-glucose is soluble; that of D-mannose is insoluble. Careful observation during the preparation of an osazone from D-mannose will reveal the formation of a white precipitate, which changes into a yellow one. The white precipitate is the hydrazone of D-mannose.

If methylphenylhydrazine is substituted for phenylhydrazine in the preparation of osazones, it is observed that only ketoses react. This reaction often serves to distinguish between aldose and ketose sugars.

The importance of osazones is that they are easily formed from reducing sugars and may be used to identify sugars. Osazones are differentiated by their microscopic appearance (see Fig. 14) and also by their melting points. The osazone of lactose, for example, has a very characteristic appearance under the microscope.

Ester Formation. Since sugars contain OH groups, they should form esters with acids. Perhaps the most important of these esters from the biochemical standpoint are those in which H_3PO_4 is the acid. It is believed that, before glucose can be utilized as a food by the animal body, it must be first converted into a phosphate.



D-Glucose

D-Glucose-6-phosphoric acid

The 6 in D-glucose-6-phosphoric acid indicates that the H_2PO_4 group it attached to the sixth carbon atom of glucose, counting from the aldehyde carbon.

Fermentation of Sugars. The word fermentation usually brings to mind the conversion of sugar into alcohol by means of yeast. Common bread yeast will ferment only sugars with three, or a multiple of three, carbon atoms. The hexose sugars D-glucose, D-mannose, and D-fructose ferment easily; D-galactose ferments with difficulty. Pentoses do not ferment with common bread yeast. The reaction taking place during alcohol fermentation may be represented by the following equation:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{Ethyl alcohol}}{2C_2H_5OH} + 2CO_2$$

This equation, however, merely represents the net results of alcoholic fermentation. In reality the process involves a long series of complicated reactions, which will be discussed in Chapter 12 on carbohydrate metabolism.

In addition to alcoholic fermentation other changes produced by microorganisms acting on carbohydrates are classed as fermentations. When milk sours, the lactic acid bacteria change the lactose of milk into lactic acid. This reaction is spoken of as lactic acid fermentation. At the present time citric acid is made commercially by the fermentation of sucrose by a fungus. We also have oxalic and butyric acid fermentations, as well as many others.

Mutarotation. In our discussion of simple sugars, up to this point, they have been considered to be straight-chain aldehyde or ketone derivatives of polyhydric alcohols. Although the simple sugars show many of the properties of aldehydes and ketones, in many respects they do not react as aldeyhdes and ketones do. For example, they do not give **Schiff's test** for aldehydes. In Schiff's test a solution of the dye fuchsin is decolorized by adding sulfur dioxide. Upon adding an aldehyde the red color is restored. A solution of an aldose sugar added to a decolorized fuchsin solution does not restore the red color, which indicates that there is no aldehyde group present. To account for the absence of a free aldehyde or ketone group in sugars new formulas are necessary. Furthermore, simple sugars exhibit an interesting property known as **mutarotation,** which can be explained on the basis of a new structure of the molecule.

If a solution of a reducing sugar is prepared and its rotation observed, it will be noticed that the rotation changes on standing. If the solution is allowed to stand for several hours, the rotation finally becomes constant. A drop of alkali will produce the constant reading immediately. This change in rotation is called mutarotation.

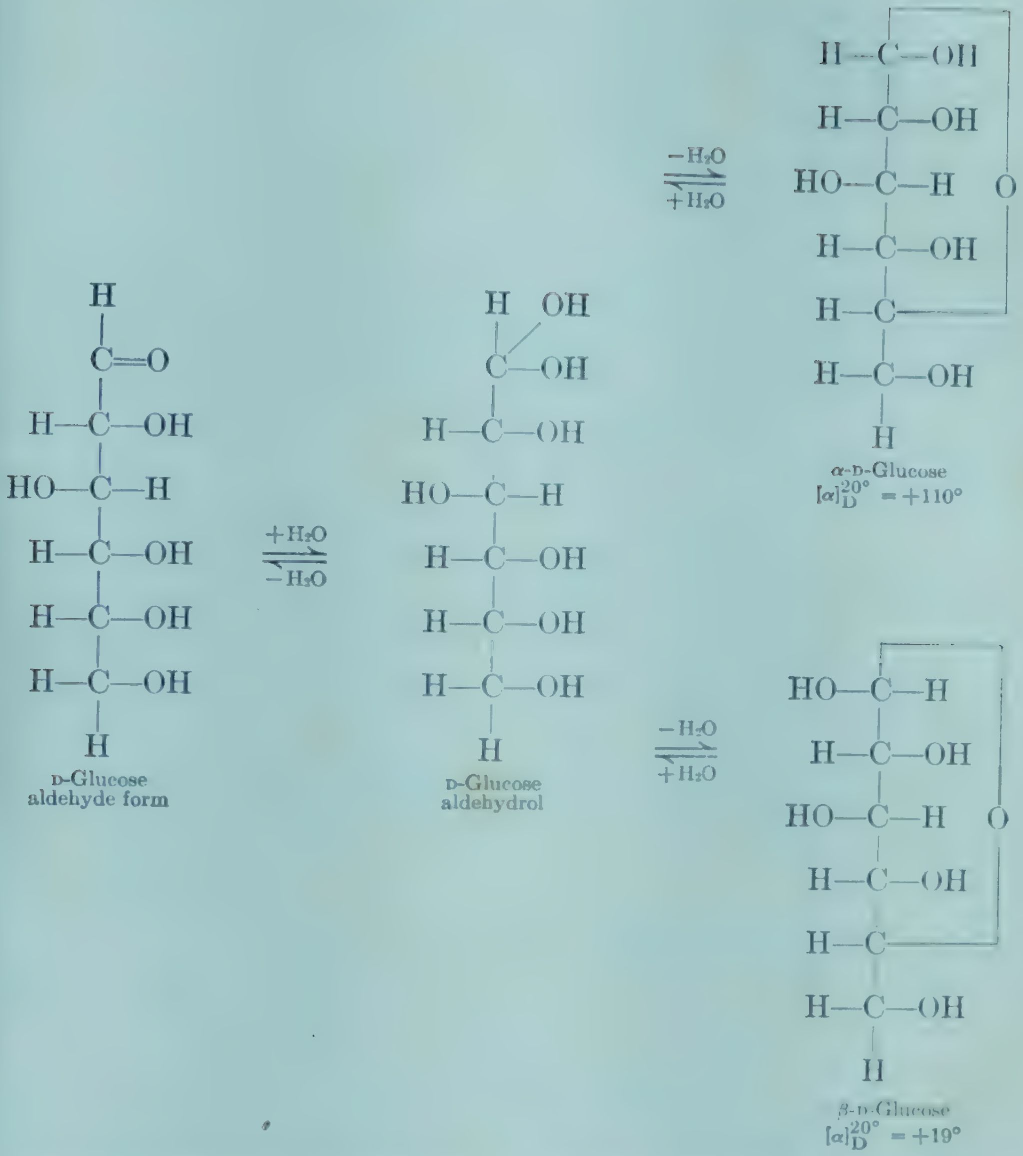
Mutarotation has been explained on the basis that reducing sugars exist in two isomeric forms, which have been called the alpha and beta forms. For D-glucose the α form has a specific rotation of +110°; the β form, of +19°. Ordinary dry glucose is mainly α-D-glucose, so that immediately after solution its specific rotation approaches +110°. As soon as α-D-glucose dissolves, some of it changes to β-D-glucose until an equilibrium mixture of the two sugars is produced when the specific rotation becomes constant at +52.5°. If β-D-glucose is the starting material, the initial specific rotation approaches +19°. Then some β-D-glucose changes to α-D-glucose, until the same equilibrium mixture is obtained, and the specific rotation again becomes constant at +52.5°.

In order to explain the existence of two varieties of D-glucose a more complicated structure for D-glucose than that presented in our previous discussion must be assumed. It is now believed that the structure of D-glucose is best represented by a formula in which the aldehyde carbon atom is linked to the fifth carbon atom through an oxygen atom. This linkage is spoken of as the 1,5-oxygen ring structure. Since with this new type of linkage the aldehyde carbon atom becomes asymmetric, it is obvious that there should be two isomers of D-glucose, namely, α- and β-D-glucose.

The origin of the 1,5-oxygen ring structure of a sugar from the aldehyde structure has been explained by assuming that the aldehyde reacts with water to form an aldehydrol where two hydroxyl groups replace the oxygen of the aldehyde group. Next a molecule of water is lost from one of these hydroxyl groups and the hydroxyl group on the fifth carbon atom, and a 1,5-oxygen ring is formed. Depending on which hydroxyl group on the first carbon atom is involved either an α or a β sugar results.

Since these formulas do not contain a free aldehyde group and since many of the reactions of sugars have been explained on the assumption that a free aldehyde group is present in a sugar, it is assumed that in a solution containing α- and β-D-glucose some free aldehyde sugar is present, probably in very small amounts, in equilibrium with the α and β forms. As the free aldehyde sugar is removed in a reaction, such as occurs with Fehling's solution, more is formed from the α and β forms until finally no more α and β sugar remains. A solution of D-glucose then should be looked upon as containing α- and β-D-glucose and a small amount of the free aldehyde form. Dry D-glucose, as found in the stockroom, is not the free aldehyde sugar, but rather α-D-glucose or β-D-glucose or a mixture of the two. As a rule the α form predominates.

The α and β forms of D-glucose may be prepared from a solution


D-Glucose aldehyde form
+H₂O
−H₂O
D-Glucose aldehydrol
−H₂O
+H₂O
α-D-Glucose
[α]$^{20°}_{D}$ = +110°
−H₂O
+H₂O
β-D-Glucose
[α]$^{20°}_{D}$ = +19°

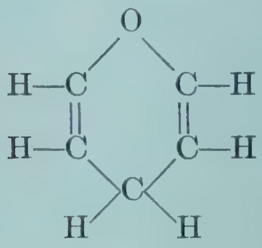
of anhydrous glucose. If anhydrous glucose is dissolved in dilute acetic acid and allowed to crystallize slowly at room temperature, α-D-glucose separates out. If anhydrous glucose is dissolved in water, glacial acetic acid is added, and the solution is heated to 100° C. for half an hour, on cooling to 98° C. β-D-glucose crystallizes out.

It will be noticed that both the α and β forms of D-glucose have five asymmetric carbon atoms. We have already said that an aldohexose has sixteen possible isomers because of the presence of four asymmetric carbon atoms. For the same reason α-D-glucose should have thirty-two possible isomers. In other words, each of the sixteen aldohexoses may exist in the α and β forms. In the formulas for α-

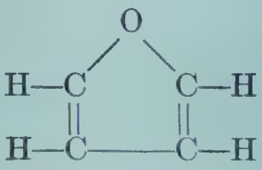
and β-D-glucose it should be noted that the hydroxyl of the potential aldehyde group is on the right in the α form and on the left in the β form.

There has been much discussion as to which carbon atoms are involved in the formation of the oxygen ring structure of a sugar. Until recently a 1,4-oxygen ring structure was considered correct. At the present time we believe that the 1,5-oxygen ring structure best accounts for the properties of the common sugars. However, it should be mentioned that the 1,4-oxygen ring structure does occur in certain sugars.

Pyranose and Furanose Formulas for Sugars. The oxide ring formulas for sugars have suggested their relationship to two heterocyclic compounds, **pyran** and **furan.**

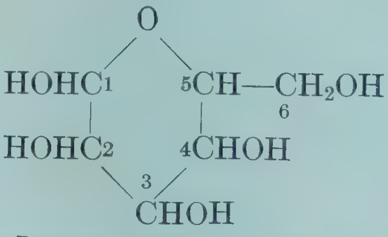


Pyran



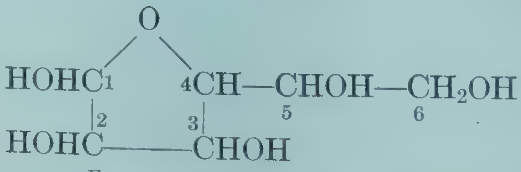
Furan

Considered as a derivative of pyran, the 1,5-oxygen ring formula for an aldohexose becomes:



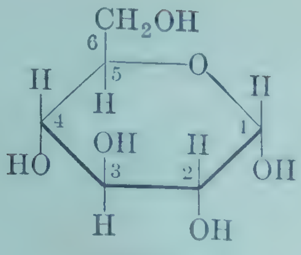
Pyranose formula for an aldohexose

Considered as a derivative of furan, the 1,4-oxygen ring formula for an aldohexose becomes:

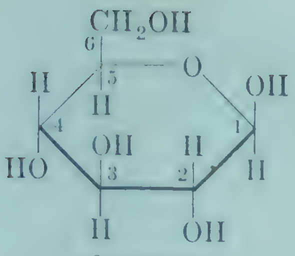


Furanose formula for an aldohexose

Haworth has suggested formulas for sugars in which the carbons in the oxygen ring are omitted. The oxygen ring is represented as a plane at a right angle to the plane of the paper. The thick lines represent the front of the ring and the thin lines the back. The Haworth formulas for α-D- and β-D-glucose are:



α-D-Glucose



β-D-Glucose

In showing the position of hydroxyl groups in Haworth formulas, it is customary to indicate the right-hand side of the molecule by directing the hydroxyl group toward the bottom of the page and the left-hand side of the molecule by directing the hydroxyl group toward the top of the page. This rule also applies to α and β sugars. In α-D aldose sugars the hydroxyl group attached to carbon-1 is directed toward the bottom of the page and in β-D sugars to the top of the page.

Since Haworth formulas for sugars are being used more and more, it is important that the student become familiar with them.

Photosynthesis. The ultimate source of energy for both plants and animals is the sun. Animals get their energy from the foods which they eat. Although animals may live on animal foods, ultimately these foods are derived from plants. A child may live on milk, but the cow under normal conditions lived on plant materials. The process by which plants build up food materials by means of sunlight is called **photosynthesis.**

The raw materials which plants use in the synthesis of carbohydrates are carbon dioxide and water. The synthesis of a hexose monosaccharide may be represented by the following equation:

$$6CO_2 + 6H_2O + \underset{\text{(677.2 Calories)}}{\text{Solar energy}} = C_6H_{12}O_6 + 6O_2$$

This reaction is made possible in plants by the pigments which are present, especially **chlorophyll.**

When an animal or plant metabolizes sugar, the photosynthetic reaction is reversed. The oxygen derived from respiration oxidizes the sugar to carbon dioxide and water with the liberation of energy, which is utilized by the body.

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \underset{\text{(677.2 Calories)}}{\text{Energy}}$$

From the preceding equation it is apparent that plants and animals have a very interesting relationship. During photosynthesis plants give off oxygen, which is essential for animal life. During respiration animals give off carbon dioxide, which is the raw material from which plants make their own food as well as ours.

From what has been said it would appear that photosynthesis is a very simple and well-understood process. This, however, is far from the truth. Many studies have been made of the mechanism of the photosynthetic process, and many theories have been advanced concerning the chemical reactions which take place during photosynthesis.

Although our knowledge is incomplete concerning the chemical changes involved in photosynthesis, certain facts appear to have been established. (1) The photosynthetic process takes place in two phases, one occurring in the dark called the **Blackman reaction** and the other occurring in the light in the presence of chlorophyll. (2) By the use of water containing isotopic oxygen (O^{18}) it has been shown that the oxygen evolved during photosynthesis comes from water and not from carbon dioxide. (3) By the use of carbon dioxide with isotopic carbon (C^{14}) it has been shown that one of the first products formed is phosphoglyceric acid.

According to Fager, Rosenberg, and Graften the first step in photosynthesis, which takes place in the dark, involves the addition of carbon dioxide to some unidentified two-carbon molecule (C_2) to form an unidentified three-carbon molecule ($C_2 \cdot CO_2$). Next, in the presence of chlorophyll and light, water is decomposed into active hydrogen, which acts as a reducing agent, and oxygen, which is evolved. After water has been activated by light the decomposition of water may continue in the dark. Thus reduction and oxygen evolution may take place both in the light and in the dark. The product formed when the unidentified three-carbon molecule ($C_2 \cdot CO_2$) is reduced is phosphoglyceric acid. The next step in the reaction involves the reduction of the carboxyl group of phosphoglyceric acid to an aldehyde giving phosphoglyceric aldehyde, which is a derivative of a triose sugar. Finally two molecules of phosphoglyceric aldehyde unite to form a hexose diphosphate, which may be converted into a hexose sugar or into a polysaccharide such as starch or cellulose.

What has just been said is presented diagrammatically as follows:

$$C_2 + CO_2 \rightarrow C_2 \cdot (CO_2) \xrightarrow[\text{in light or dark}]{H} \text{Phosphoglyceric acid} \xrightarrow[\text{in light or dark}]{H}$$

$$\text{Triose phosphate} \rightarrow \text{Hexose diphosphate} \begin{array}{l} \nearrow \text{Hexose} \\ \searrow \text{Polysaccharides} \end{array}$$

It should be pointed out that the above description of the photosynthetic process is undoubtedly an oversimplification. When details of the process finally become known they will likely reveal a much more complicated process than that described here. In the chapter on carbohydrate metabolism which follows later in this book, the details of how sugars are broken down in the body are given. It is quite possible that when the details of photosynthesis are known they may prove to be the reverse of those reactions occurring when sugars are broken down for energy in the body. Considerable evidence is accumulating to indicate that this is the case.

Although the term photosynthesis is sometimes restricted to the synthesis of carbohydrates, it should be mentioned that proteins and other nitrogenous plant compounds also owe their origin to the photosynthetic process. It is likely that carbohydrates are an intermediate stage in the synthesis of fat. The process involves a reduction of the carbohydrate and a condensation. Photosynthesis is thus closely associated with the synthesis of all classes of organic compounds in the plant kingdom.

The Monosaccharides

D-Glucose. D-Glucose is the most important of the monosaccharides. Commercially it is called **dextrose**, a name derived from the fact that it is dextrorotatory, its specific rotation being +52.5°. (See Table 3.)

TABLE 3

SPECIFIC ROTATION OF CARBOHYDRATES

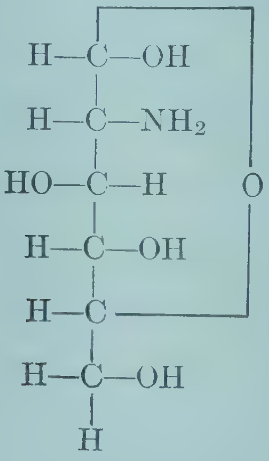
Carbohydrate	Specific Rotation	Carbohydrate	Specific Rotation
L-Arabinose	+105.0°	Sucrose	+ 66.5°
D-Xylose	+ 19.0°	Lactose	+ 55.5°
D-Ribose	− 21.5°	Maltose	+136.0°
D-Glucose	+ 52.5°	Cellobiose	+ 34.6°
D-Fructose	− 92.0°	Raffinose	+104.5°
D-Mannose	+ 14.6°	Dextrin	+195.0°
D-Galactose	+ 80.5°	Starch	+196.0°
		Glycogen	+197.0°

Sometimes it is referred to as **grape sugar**, because it is present in high concentration in grapes. It is the most widely distributed of the sugars, being found in most plants and in the blood of animals. Normally human blood contains about 100 mg. of D-glucose per 100 ml. In the disease known as **diabetes** the concentration in the blood is increased. When it reaches a level of about 160 mg. per 100 ml., it is excreted in the urine, so that a positive test for sugar in the urine is usually an indication of diabetes. Since the kidney concentrates the urine, as much as 10 per cent of D-glucose may be found in the urine in diabetic conditions.

Commercially D-glucose is made by hydrolyzing starch with acid. In this country corn starch is usually used, and the product is put on the market as **corn syrup**. Corn syrup usually contains some maltose and dextrins which result from the incomplete hydrolysis of the starch, and sucrose may be added to increase the sweetness. The dry sugar which is also a commercial product is called **cerelose.**

Corn syrup has wide usage commercially and in the home. In infant feeding it is often used as the carbohydrate added to modify cow's milk. Since D-glucose is the sugar of the blood, it requires no digestive changes in order to be utilized by the body. When a patient is unable to take food by mouth, D-glucose solutions may be given by rectum or by injection directly into the blood stream.

An interesting derivative of D-glucose called **glucosamine** is found in **chitin,** a constituent of the shell of lobsters, in the cartilages of other animals, and in the mucin of the saliva. Glucosamine is an amino sugar in which one of the OH groups of the sugar is replaced by NH_2. Chitin is a glucosamine polysaccharide, in which the glucosamine is present as an acetyl derivative with an H of the NH_2 group replaced by the acetyl radical. In cartilage and mucin the glucosamine is a part of a glycoprotein molecule. Thus in animals glucosamine is found not as a free substance but as a part of more complex molecules.



α-D-Glucosamine

D-Fructose. The most important fact to remember about D-fructose is that it is a **ketose sugar.** In fact, it is the only ketose sugar commonly encountered in biochemistry. It will be noted in Table 4 that it is the sweetest of the common sugars. If the sweetness of sucrose is considered as 100, D-fructose has a relative sweetness of 173.3. The sweetness of many fruits is due to the presence of D-fructose and for this reason it is sometimes called **fruit sugar.** It is also called **levulose** because of the fact that in solution it is strongly levorotatory, its specific rotation being −92° at 20°C. A point which should be emphasized in this connection is that the rotation of D-fructose solutions is greatly affected by temperature changes. For every 2°C. rise in temperature the

TABLE 4

THE RELATIVE SWEETNESS OF COMMON SUGARS

Sugar	Relative Sweetness	Sugar	Relative Sweetness
Fructose	173.3	Maltose	32.5
Sucrose	100.0	Galactose	32.1
Glucose	74.3	Raffinose	22.6
Xylose	40.0	Lactose	16.0

specific rotation of D-fructose is reduced 1°. Thus in analyzing by means of a polariscope sugar solutions containing D-fructose, the temperature at which readings are made must be known.

D-Fructose is widely distributed in nature, especially as a constituent of the disaccharide sucrose. About one-half the sugar of honey is D-fructose; inulin, an important polysaccharide, gives only D-fructose on hydrolysis. D-Fructose may be prepared from hydrolyzed inulin or sucrose. Since hydrolyzed sucrose is a mixture of D-glucose and D-fructose and since it is difficult to separate these two sugars, it is easier to prepare pure D-fructose from inulin.

Since D-fructose is the only ketose sugar ordinarily encountered in biochemical work, a test for a ketose sugar may be interpreted as a test for D-fructose. There are two such tests commonly used, namely, **Seliwanoff's test** and **osazone formation with methylphenylhydrazine.** Seliwanoff's reagent, a solution of resorcinol in HCl, on boiling with a solution of a ketose sugar gives a red color and a brown precipitate. Methylphenylhydrazine forms an osazone with a ketose but not with an aldose sugar. D-Fructose gives the same osazone with phenylhydrazine as does D-glucose; hence these two sugars must have the same arrangement of groups on the last four carbon atoms.

D-Fructose has the same food value as D-glucose. The liver can convert D-fructose into D-glucose, the sugar which is found in the blood and from which glycogen is made.

D-Mannose. D-Mannose is not found free in nature but is rather widely distributed in the form of polysaccharides called **mannans.** Vegetable ivory, a product which is obtained from ivory nuts, and from which buttons are often made, is a polysaccharide that gives D-mannose on hydrolysis and is the material from which D-mannose is most easily prepared. A trisaccharide containing two molecules of mannose and one of glucosamine has been found in egg white.

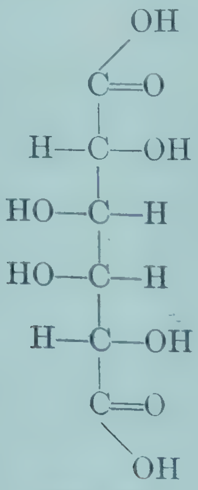
D-Mannose differs from D-glucose in the arrangement of groups on the second carbon atom. It therefore gives the same osazone as does D-glucose, but the hydrazone is different. The specific rotation of D-mannose is +14.6°. An interesting characteristic of D-mannose is that it has a bitter, rather than a sweet, taste.

On reduction D-mannose gives the polyhydric alcohol D-mannitol, which is rather widely distributed in nature, being found in silage, certain vegetables, and pineapples. Commercially D-mannitol is prepared by the electrolytic reduction of D-glucose, a process whereby D-glucose is converted to D-mannose, which on reduction gives D-mannitol. On nitration D-mannitol is converted into an important explosive.

D-Galactose. The most common occurrence of D-galactose is in lactose, the sugar of milk, a disaccharide composed of D-glucose and D-galactose. It also occurs as a part of complex molecules in pectin, gums, mucilages, and agar-agar, a mucilage made from seaweed and used in preparing solid bacteriological culture media. Agar-agar contains a polysaccharide which gives D-galactose on hydrolysis. In the animal body D-galactose is found in the brain and nervous tissue in the form of glycolipids.

The D-galactose necessary for the synthesis of lactose in milk is made in the mammary gland from D-glucose. It would appear that lactose is important in an infant's diet, since D-galactose is a constituent of such important tissues as those of the nervous system. However, an infant is undoubtedly able to synthesize sufficient D-galactose from other sugars in its diet to meet its requirements.

D-Galactose has a specific rotation of +80.5°. Since it differs in structure from D-glucose on the fourth carbon atom, the two sugars form different osazones. On oxidation with HNO_3 D-galactose forms a dicarboxylic acid called **mucic acid** and corresponding to saccharic acid, which is obtained when D-glucose is similarly oxidized. Mucic acid differs from saccharic acid in that it is insoluble and forms characteristic crystals. The formation of insoluble mucic acid crystals on oxidation with HNO_3 is one of the best tests for D-galactose. Mucic acid has the following formula:



Mucic acid

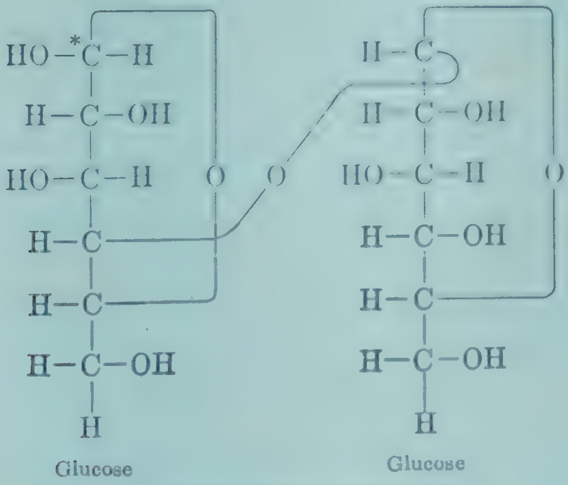
The Disaccharides

The disaccharides are a group of compound sugars composed of two monosaccharides linked together through the loss of water. They are all regarded as being derived from monosaccharides, having the oxide ring structure. The linkage is always through the potential aldehyde or ketone group of at least one sugar and sometimes of both.

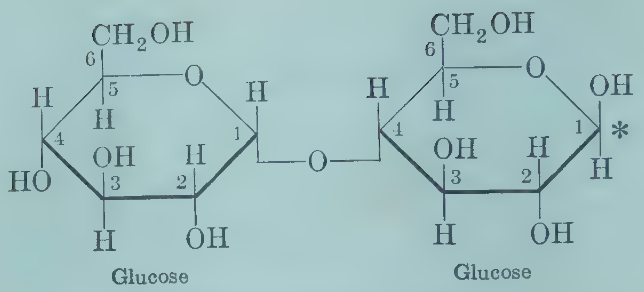
Disaccharides may be divided into two classes, namely, those which reduce Fehling's solution and those which do not. If the potential aldehyde or ketone group of only one of the sugars is involved in the linkage, the resulting sugar will reduce Fehling's solution. If the potential aldehyde or ketone groups of both sugars are involved, the sugar will not reduce Fehling's solution. In the first case the sugar still has one reducing group; in the second, no reducing group is present. Reducing disaccharides exhibit most of the properties of monosaccharides. They form osazones and show mutarotation. The important reducing disaccharides are maltose, cellobiose, and lactose; sucrose is the only important nonreducing disaccharide.

Maltose. Maltose is the most common reducing disaccharide. In nature it is found not as a free substance but as a constituent of the polysaccharides, starch, and glycogen. Starch is found in all green plants, and glycogen is the form in which carbohydrates are stored in the animal body.

Since maltose is a reducing sugar, it shows mutarotation and therefore exists in α and β forms. The common variety of maltose is the β form. The straight chain and Haworth formulas for β-maltose are as follows:



β-Maltose (β-D-glucose-4-α-D-glucoside)



Glucose Glucose

Haworth formula for β-maltose

The formula for α-maltose has the positions of the H and OH groups on the starred carbon atom reversed.

The specific rotation of β-maltose is +118° and of α-maltose +168°; after mutarotation the equilibrium mixture has a specific rotation of +136°.

Derivatives of glucose or any other sugar in which the H of the OH group, attached to the potential aldehyde carbon, is replaced by some other group are called **glycosides.** If the sugar is glucose the derivative may be called a **glucoside.** Hence maltose may be looked upon as a glucose glucoside. The chemical name for β-maltose is β-D-glucose-4-α-D-glucoside. This name is descriptive of the structure of maltose. It indicates that maltose is composed of one molecule of β-D-glucose and one of α-D-glucose. In the straight-chain formula the first glucose molecule is beta because the OH groups on the starred carbon atom is to the left. The 4 indicates that the second glucose molecule is attached to the fourth carbon atom of the first sugar. The second glucose molecule is alpha because the OH group on the top carbon atom involved in the linkage of the two sugars is on the right. The second D-glucose is considered the nucleus of the glucoside.

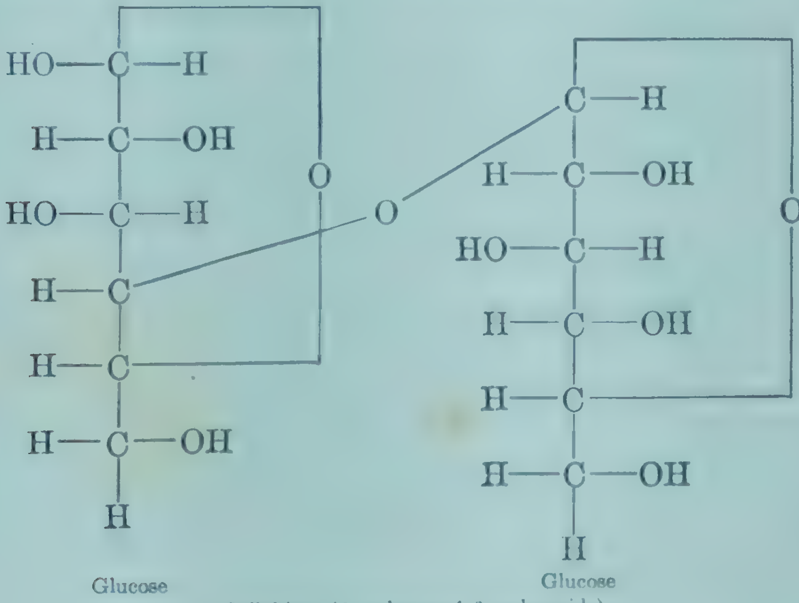
Two types of glycosides are recognized, namely, alpha and beta, depending upon whether the sugar forming the nucleus of the glycoside is of the α or the β variety. All α-glycosides are hydrolyzed by **maltase** and all β-glycosides by the enzyme **emulsin.** Thus we have an easy method of distinguishing between α- and β-glycosides, which is of great value in determining the structure of disaccharides. Since maltose is hydrolyzed by maltase and not by emulsin, it must be an α-glycoside.

Maltose derives its name from the fact that it is the product formed from starch in the malting process, in which barley is allowed to sprout under controlled conditions. During the sprouting, enzymes capable of converting starch into maltose are produced. The sprouted barley is known as malt. If malt is extracted with water and the solution is concentrated, a syrup which is composed mainly of maltose results. Malt is used in the manufacture of beer and other malted beverages.

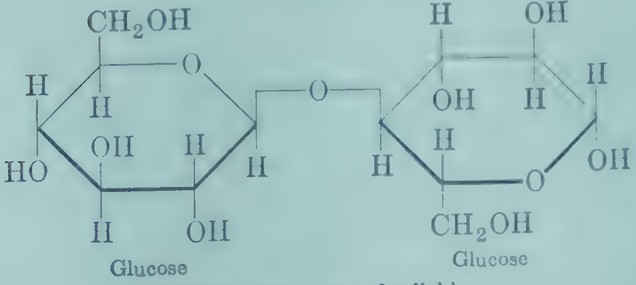
When added to a mash made of grains its enzymes convert the starch present to maltose. Then yeast, which contains the enzyme maltase, converts the maltose to glucose. Finally yeast, by means of several enzymes, converts the glucose to ethyl alcohol and carbon dioxide.

In the animal body the **amylases** of the saliva and of the pancreatic juice hydrolyze starch to maltose. Maltose is hydrolyzed into two molecules of glucose by the enzyme **maltase,** which is found in the intestinal juice. Maltose forms a characteristic osazone, which is different from that of glucose.

Cellobiose. We have just learned that, when starch is partially hydrolyzed, the disaccharide maltose is formed. When cellulose is partially hydrolyzed, cellobiose is the resulting disaccharide. Although both maltose and cellobiose yield D-glucose on hydrolysis, they are quite different sugars, and it is of interest to know in what respect they differ. Maltose is hydrolyzed by maltase, whereas cellobiose is not. Cellobiose, however, is hydrolyzed by emulsin. Therefore cellobiose must be a β-, rather than an α-, glucoside. This appears to be the only difference



β-Cellobiose (β-D-glucose-4-β-D-glucoside)



Haworth formula for β-cellobiose

between maltose and cellobiose; whereas maltose is β-D-glucose-4-α-D-glucoside, cellobiose is β-D-glucose-4-β-D-glucoside.

In the Haworth formula for β-cellobiose it will be noted that the glucose molecule to the right has been rotated 180°. This is done in order to bring the OH group on the fourth carbon atom of this glucose molecule to the same side of the molecule as the OH group on the first carbon atom of the β-D-glucose molecule to the left. Thus all groups tied to carbon in the glucose molecule to the right are reversed.

It is interesting to note how important slight differences in chemical structure may be from the biochemical standpoint. Starch, which contains the α-glucosidic structure, is one of our most important foods. Cellulose, which contains the same fundamental units as starch but is built up through β-glucosidic linkages, is of no value as a food because we cannot digest it.

Lactose. The sugar of milk, called lactose, is made up of one molecule of glucose and one of galactose. It forms a very characteristic osazone (see Fig. 14) and exhibits mutarotation, as it exists in solution in two forms, alpha and beta. The specific rotation of the equilibrium mixture is +55.5°.

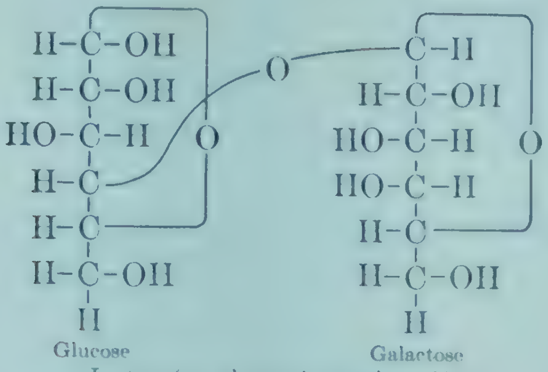
When lactose crystallizes from a cold solution, the α variety, which is not very soluble, is obtained. It has a specific rotation of +89.4°. The rough consistency in "sandy" ice cream is due to the crystallization of α-lactose. Sharp has found that, if lactose is allowed to crystallize from a saturated solution at a temperature above 94°C., **β-lactose** is obtained. β-Lactose has a specific rotation of +35°, is more soluble at room temperature than α-lactose, and because of its greater solubility appears to be sweeter. Important applications for β-lactose have been found in infant feeding and in certain dietaries.

Lactose is present in cow's milk to the extent of about 4 per cent and is obtained by evaporating milk whey. In the animal body lactose is synthesized in the mammary gland. During lactation it may occur in the urine; therefore a positive test for sugar, under these conditions, does not necessarily mean diabetes.

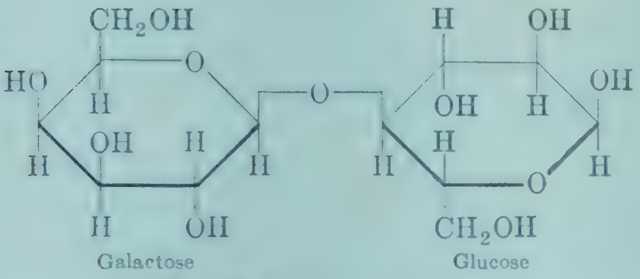
Lactose is not so easily fermented as many of the other sugars and consequently is of considerable value in bacteriology for differentiation purposes. The fact that lactose does not ferment readily makes it an ideal constituent of milk. If milk contained glucose in place of lactose, it would not keep so well and would ferment rapidly in the stomach, causing digestive disturbances in infants.

The main function of lactose in milk, however, may be to furnish the galactose necessary for the synthesis of the glycolipids of the brain and nervous tissue. Lactose is hydrolyzed by the enzyme **lactase** into its constituent monosaccharides. It is also hydrolyzed by **emulsin**, indicating that it is a β-glycoside.

The formula for lactose is:



α-Lactose (α-D-glucose-4-β-D-galactoside)



Haworth formula for α-lactose

Lactose is a galactoside, being the α-D-glucose derivative of β-D-galactose. The fourth carbon atom of glucose is involved in the linkage of the two sugars.

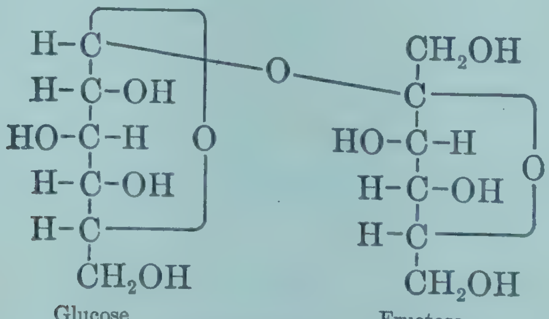
Sucrose. Sucrose is commonly called **cane sugar** and sometimes **saccharose.** It is the ordinary sugar which is used in cooking and on the table. It is very widely distributed in nature, being found in sugar cane, sugar beets, maple sugar, and sorghum syrup. At the present time about one-third of our cane sugar comes from sugar beets. It has sometimes been said that beet sugar is inferior to cane sugar for certain types of cooking, such as jelly making. Experiments have shown that there is no ground for such a contention. Cane sugar and beet sugar are both sucrose and have equal value as food. When sucrose is heated to 160° C., it melts and on cooling forms a glassy solid called **barley sugar.** When it is heated to 210° C., decomposition occurs, yielding a brown product known as **caramel.**

Cane sugar is a disaccharide made up of molecules of α-D-glucose and of β-D-fructose, the linkage involving the potential aldehyde and ketone groups of the two sugars. Its chemical name is α-D-glucose-1-β-D-fructofuranoside. Furanoside indicates that the oxygen ring in the fructose molecule is between the second and fifth carbon atoms; in other words, fructose as it occurs in sucrose has a furanose or a 2,5-oxygen ring structure. In naming disaccharides the pyranose or 1,5-oxygen ring structure is assumed unless otherwise indicated. Since sucrose does not contain a potential aldehyde or ketone group, it does not reduce Fehling's solution, form osazones, or show mutarotation. The reason sucrose does not show mutarotation is that it does not exist in α and β forms.

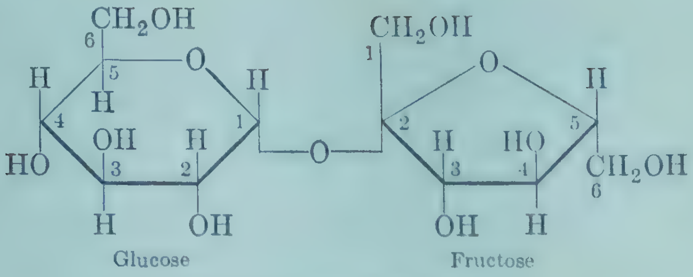
The fact that sucrose in solution exists in a single form accounts for its ready crystallization. Other sugars, such as glucose, do not crystallize so readily, because they exist in solution as a mixture of the α and β forms. It is a well-known fact that solutions of single compounds crystallize better than solutions of mixtures. When sucrose is used in candy making, it often crystallizes in large crystals, giving a product of poor quality. Crystallization may be prevented by adding other sugars in the form of corn syrup or by hydrolyzing some of the sucrose during the cooking process through the addition of a small amount of acid, such as vinegar or cream of tartar.

Sucrose is very easily hydrolyzed by acid, much more readily than maltose or lactose. In jelly making it has been estimated that one-half of the sucrose added is hydrolyzed during the cooking process by the acids present in the fruit juices. In addition to preventing crystallization, another advantage in hydrolyzing sucrose is that the mixture of sugars resulting from hydrolysis is 30 per cent sweeter than the original sucrose. Sucrose may be hydrolyzed by the enzyme **sucrase,** often called **invertase,** as well as by acid. In making chocolate creams invertase is often incorporated into the cream fillings, thereby causing a slow hydrolysis of the sucrose present which prevents crystallization and increases the sweetness with age.

The formula for sucrose is as follows:



Sucrose (α-D-glucose-1-β-D-fructofuranoside)



Haworth formula for sucrose

The specific rotation of sucrose is +66.5°. After hydrolysis the specific rotation of the mixture of sugars is −19.84°. In other words, after hydrolysis the rotation is inverted. Therefore the process has been called **inversion**, and the mixture of sugars formed, **invert sugar.** The reason for the levorotation of invert sugar is that fructose is more strongly levorotatory than glucose is dextrorotatory. The equation expressing the inversion of cane sugar is:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underbrace{\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}}_{\text{Invert sugar}}$$

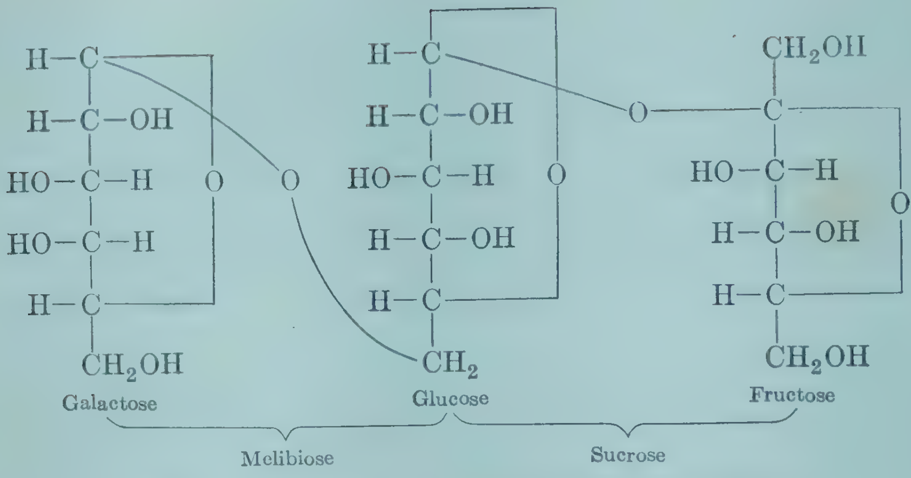
When a 50 per cent solution of sucrose is treated with $Ca(OH)_2$ and filtered, a solution known as **viscogen** is formed. When this is added to ordinary cream in small amounts, it becomes possible to whip the cream.

Trisaccharides

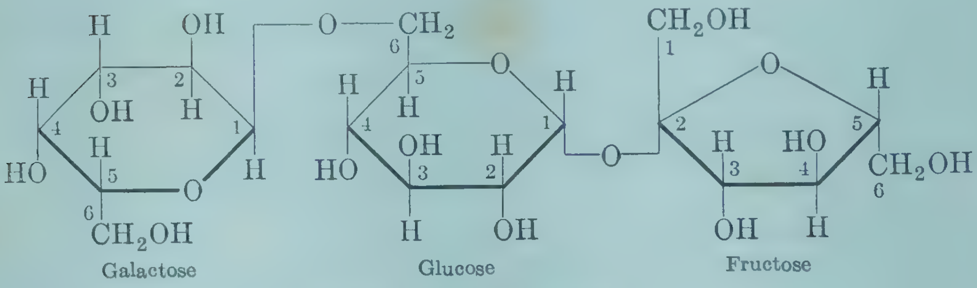
The only important trisaccharide is **raffinose,** which is found in cottonseed meal, soy beans and sometimes in sugar beets. It is a nonreducing sugar and has a specific rotation of +104°. When present in sugar beets as it often is after a frost, it interferes with the polariscopic determination of sucrose and corrections must be made for it.

Raffinose is composed of galactose, glucose, and fructose and may be hydrolyzed by strong acid into these three sugars. Weak acid hydrolyzes raffinose into fructose and a disaccharide called **melibiose,** which is isomeric with lactose. Emulsin, which contains an α-galactosidase as well as a β-glycosidase, hydrolyzes raffinose into sucrose and galactose.

Raffinose is of no value as a food unless it is first hydrolyzed. Hydrolysis may occur in the digestive tract as a result of the action of the microorganisms present.



Raffinose (α-D-galactose-1,6-α-D-glucose-1,2-β-D-fructofuranoside)



Haworth formula for raffinose

Polysaccharides

The polysaccharides are complex carbohydrates of high molecular weight composed of many monosaccharide units combined through the loss of molecules of water. They differ from most sugars in that they do not reduce Fehling's solution and are not sweet. In general they are insoluble in water, but many of them will give colloidal dispersions under certain conditions. Included in this group are many of the most important carbohydrates.

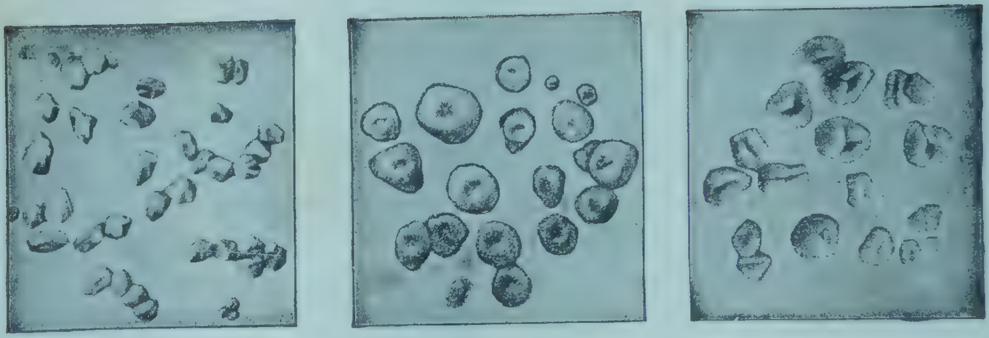
Pentosans. Polysaccharides that on complete hydrolysis yield only pentoses are called pentosans. The terms **xylan** and **araban** are used to indicate pentosans which give xylose and arabinose, respectively, on hydrolysis. Xylan is found in wood, corncobs, straw, bran, and cottonseed hulls. Araban is found in gums, such as gum arabic and cherry gum, in mucilages, and in fruit juices.

Concerning the physiological importance of pentosans little is known. They are strictly plant products, and one valuable property is that they are highly hygroscopic and aid plants in retaining water. There are no enzymes in the digestive tract capable of hydrolyzing them. In animals, however, considerable amounts of pentosans disappear from food as it passes through the digestive tract. It is thought that this is due to bacterial action and that any benefit which the animal body derives from pentosans is thus indirect. Certain microorganisms can ferment pentoses, giving rise to such products as acetic and lactic acids, which may be absorbed by the animal and utilized as food. Also we know that pentoses form a part of some very important compounds in the body, such as nucleic acid found in the nuclei of cells and in certain compounds associated with biological oxidations and reductions. Since these compounds are so important, it appears that the body must be capable of synthesizing pentoses, possibly from hexoses, because the supply of pentoses could hardly be left to chance production by microorganisms in the intestinal tract.

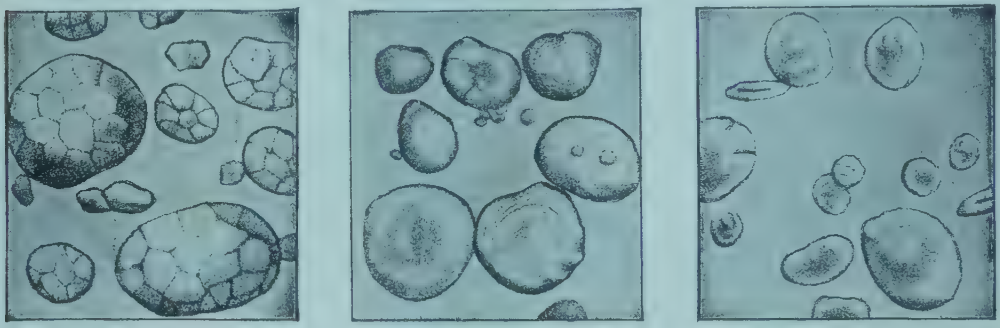
Hexosans. The hexosans are polysaccharides that give only hexoses on hydrolysis. They are subdivided into several groups depending on which hexose monosaccharide they give on hydrolysis. Those that give only glucose are called glucosans; those that give only fructose, fructosans; and those that give only mannose, mannosans. Some of the most important carbohydrates are found in this group.

Glucosans. STARCH. Starch, which is sometimes called **amylum,** is the most important source of carbohydrate in the diet. It occurs in most green plants as a reserve food supply. The principal sources of starch are cereal grains, beans, peas, and tubers, such as potatoes. Green fruits, such as apples and bananas, contain much starch, which changes to sugar as the fruit ripens. In cereals, sugars appear first, being changed to starch as the grain matures. This is especially true in sweet corn, which is quite sweet in the early stages of development but loses its sweetness as it matures.

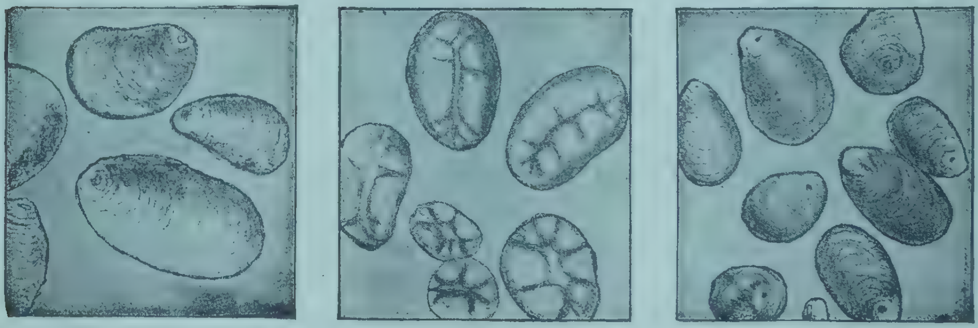
As found in plants, starch occurs in the form of granules, whose size and form are quite characteristic for each plant. In fact, the source of a starch can usually be determined by the microscopic appearance of its granules. (See Fig. 15.) This fact is often utilized in testing for adulteration in foods. Starch granules are not made up of a homogeneous material; they appear to be composed of at least two distinct substances, namely, **amylose** and **amylopectin.** These two substances have different properties, amylose being water-soluble and more easily hydrolyzed than amylopectin. Starch contains small amounts of phosphorus, which appears to be present in the amylopectin fraction as an ester of phosphoric acid. Usually small quantities of fatty acids are present in the amylose fraction. Since these fatty acids can be removed by a suitable solvent such as methyl alcohol it is thought that they are not chemically combined with the starch but are present as adsorption complexes.



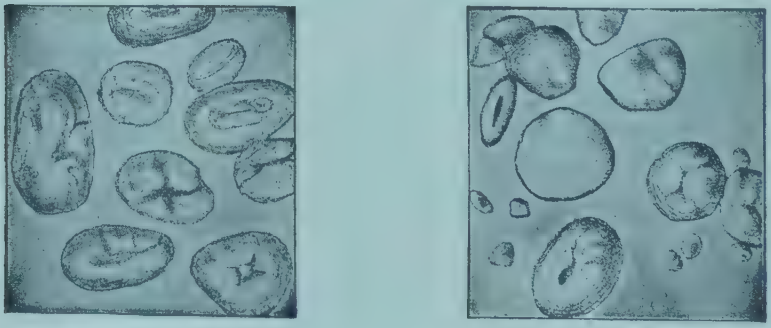
Buckwheat Maize Rice



Oat Barley Rye



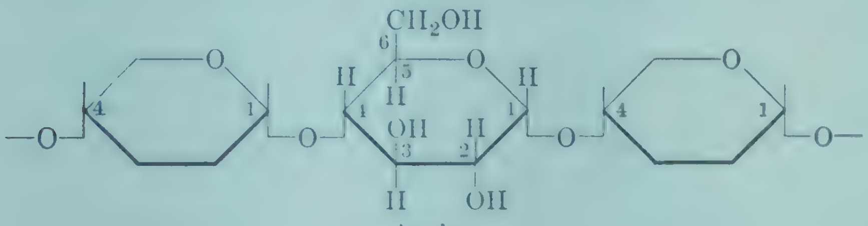
Potato Bean Arrowroot



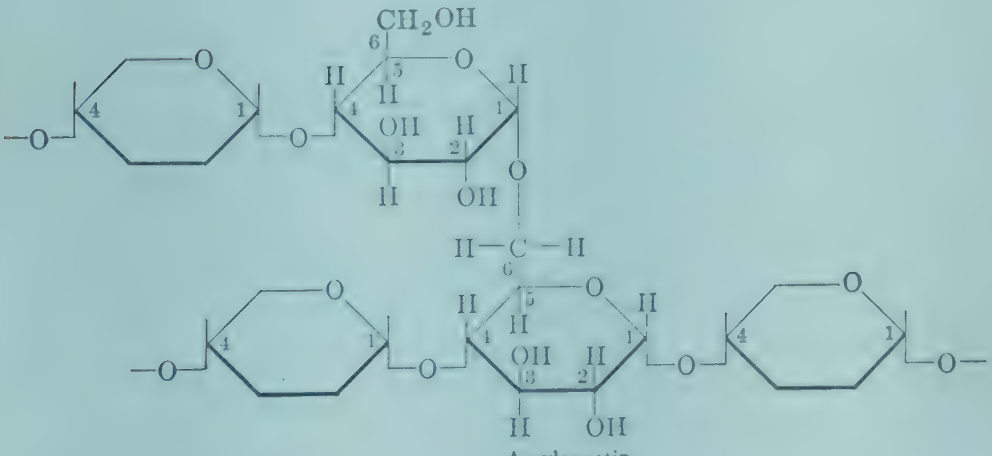
Pea Wheat

Fig. 15. Starch granules from various sources. (After Leffmann and Beam.) From *Practical Physiological Chemistry* by Hawk, Oser, and Summerson. Courtesy of the Blakiston Co.

Concerning the chemical structure of starch our knowledge is not complete, but several interesting facts are known. The amylose fraction is composed of long chains of about three hundred α-D-glucose molecules linked together by α-1,4-glucosidic linkages as in maltose. The amylopectin fraction is a branched-chain molecule in which the main chain appears to be composed of twenty-four to thirty glucose molecules. Attached to this central chain are many similar side chains; thus enormous molecules are formed which have been estimated to be composed of two thousand to three thousand glucose units and to have molecular weights as high as 500,000. (See Fig. 16.)



Amylose



Amylopectin

Fig. 16. Showing the structure of amylose and amylopectin. Amylose is linear with glucose units united through α-1,4-glucosidic linkages. Amylopectin has many chains similar to amylose united to each other through α-1,6-glucosidic linkages.

The side chains are attached to the main chain by α-1,6-glucosidic linkages involving the hydroxyl group on carbon-6 of glucose units in the main chain.

Starch can be completely hydrolyzed to maltose by enzymes. Three enzymes are involved in the process, namely α- and β-amylase and α-1,6-glucosidase. α-Amylase acts on the amylopectin fraction of starch, splitting out dextrin molecules composed of about six glucose molecules, surrounding α-1,6-glucosidic linkages which it cannot hydrolyze. Only small amounts of maltose are produced by α-amylase even though it is capable of splitting α-1,4-glucosidic linkages.

β-Amylase attacks α-1,4-glucosidic linkages, forming maltose. Thus amylose is completely hydrolyzed to maltose. With amylopectin only about 50 per cent of the molecule is converted into maltose. Maltose molecules are split off from side chains until α-1,6-glucosidic linkages are reached, leaving so-called "limit dextrin" molecules.

α-1,6-Glucosidase attacks the α-1,6-glucosidic linkages in dextrins, forming smaller molecules which may be hydrolyzed by β-amylase to maltose. Commercial preparations of starch-splitting enzymes, called **diastases,** contain all three enzymes and can therefore hydrolyze starch completely to maltose.

In many substances, especially plant materials, the starch-splitting enzymes occur along with maltase and are difficult to separate from it. If maltase is present, the end product is glucose. If starch is hydrolyzed by acid, glucose is the final product.

Starch is not fermented by yeast. In bread making, amylases in the flour first convert the starch to maltose. Maltase present in yeast next converts maltose to glucose. The glucose is then fermented to carbon dioxide and alcohol by other enzymes present in yeast.

Starch is formed in plants from glucose-1-phosphate by the action of the enzyme **phosphorylase.** Synthetic starch has been prepared in the laboratory by the action of phosphorylase, prepared from potatoes, on glucose-1-phosphate.

In connection with the chemical reactions of starch it should be mentioned that starch does not reduce Fehling's solution or form osazones, indicating that few free aldehyde groups are present. Its most characteristic reaction is the formation of a blue compound with iodine. This compound is stable only in cold solution. On heating, the blue color disappears, but it returns again on cooling. Amylose gives a more intense blue color with iodine than does amylopectin.

When a suspension of starch in cold water is added to boiling water the granules swell and a starch paste is formed. Soluble starch is made by treating starch with cold dilute hydrochloric acid.

In studying the rate of hydrolysis of starch by acids or enzymes, the iodine test is often used to tell when all the starch has been hydrolyzed. As starch is hydrolyzed a point is reached, called the **achromatic point,** where it no longer gives a color with iodine.

Starch is a very important compound commercially. In such forms as potatoes and flour it is one of our chief sources of food. Large quantities of grain and potatoes are used in the manufacture of alcohol. Corn starch is used for starching clothes. There is a high percentage of starch in baking powder, where its purpose is to keep the active ingredients apart and from reacting before use and to serve as a diluting agent, thereby making it easier for the housewife to measure the correct amount. Rice starch is a common ingredient of face powders. Arrowroot starch is used in the manufacture of tapioca.

DEXTRIN. The dextrins are a group of glucose polysaccharides which are intermediate in the hydrolysis of starch to maltose. Three kinds of dextrins, differing in their complexity, are recognized. The most complex variety is called **amylodextrin**, a name which indicates its similarity to starch. It gives a bluish red color with iodine. A less complex variety is called **erythrodextrin.** *Erythro* means red, and erythrodextrin is so called because of the red color it gives with iodine. The third variety is called **achroödextrin** because it gives no color with iodine. Greek *achromos* means without color.

Most commercial preparations of dextrin reduce Fehling's solution, the amount of reduction depending on the degree of degradation that the starch has undergone in their preparation. The large molecules of amylodextrin do not reduce Fehling's solution, but the smaller molecules of achroödextrin do.

Dextrin may easily be made from starch by heating, preferably in an autoclave. It is soluble in water and is precipitated from water solution by the addition of alcohol. We are not certain of the number of glucose units present in the various varieties of dextrin, but they range in complexity between starch and maltose.

Dextrin is used quite extensively in the manufacture of cheap adhesives because of its sticky properties when wet. Such adhesives, often spoken of as British gum, are used on postage stamps and envelopes. Many of the common mucilages and pastes on the market are made largely of dextrin. In the textile industry cotton cloth is sized with dextrin before the pattern is printed. In making candy dextrin is often added to give smoothness to the product. In baking bread much of the starch in the crust is converted into dextrin. Many of the toasted breakfast foods on the market, such as corn flakes, contain considerable quantities of dextrin produced by the heating process. In nutrition studies on small animals dextrin is often used as the source of carbohydrate in the diet because it contains no vitamins. In infant feeding a product known as **Dextri-maltose** is often used as the carbohydrate added to modify cow's milk. It is a combination of dextrins and maltose resulting from the partial hydrolysis of starch.

GLYCOGEN. Glycogen, often called **animal starch,** is a glucose polysaccharide found in animal tissue and is the storage polysaccharide of animals, corresponding to the starch found in green plants. It occurs especially in the liver and muscles, but most animal tissues contain some. Oysters contain considerable quantities of glycogen. The human liver usually contains from 1.5 to 4.0 per cent of glycogen and on a high carbohydrate diet may contain as much as 10 per cent. Muscles contain from 0.5 to 1.0 per cent. Horse muscle contains more, and this fact is utilized in testing for the adulteration with horse meat of such meat products as sausage. The sweetness of liver and horse meat is due to their high percentage of glycogen. The occurrence of glycogen is not confined to the animal kingdom, since it has been isolated also from sweet corn, yeasts, and molds.

The chemical structure of glycogen is similar to that of starch as the glucose molecules are united by α-glucosidic linkages. On hydrolysis by the amylases, glycogen gives maltose, as does starch. The glycogen molecule is thought to be composed of a branched chain similar to the amylopectin fraction of starch, but the individual chains are not so long as those in starch. Whereas the individual chains in the starch molecule are thought to contain from twenty-four to thirty glucose units, in glycogen they are believed to contain from twelve to eighteen. The molecular weight of the glycogen molecule is thought to be about one-half that of the starch molecule.

In water glycogen forms a colloidal solution which is dextrorotatory. Its specific rotation is +196.0°. With iodine it gives a mahogany-red color, which is intensified if salt is present. Glycogen does not reduce Fehling's solution or form osazones. On acid hydrolysis it first gives dextrin, then maltose, and finally glucose.

Glycogen may be prepared from liver by treatment with 30 per cent NaOH solution. In the presence of strong alkali liver tissue is destroyed, leaving unchanged the glycogen, which may be precipitated from the solution with alcohol. This is an excellent example of the stability of polysaccharides to alkali.

CELLULOSE. Normal cellulose is a glucose polysaccharide which is perhaps the most widely distributed and most important industrially of all the carbohydrates. It occurs especially in the woody parts of plants and is responsible for the structure of plants, much as protein material is responsible for the structure of animals.

Chemically cellulose is similar in structure to starch but differs in that the glucose units are β-D-glucose instead of α-. In other words, cellulose has a β-glucosidic structure, whereas starch has an α-glucosidic structure. On partial hydrolysis starch gives the disaccharide maltose, but under similar conditions cellulose gives cellobiose.

The arrangement of the glucose molecules in cellulose is linear as in the amylose fraction of starch; there are no branched chains present.

These chains may be present as single chains or as bundles of parallel chains. As to size, the cellulose molecule has been variously estimated to be made up of one hundred to two thousand glucose units.

Although cellulose is composed of glucose, it has no value as a food for man, because there are no enzymes in his digestive tract capable of hydrolyzing it. However, cellulose has a function in food in that it supplies bulk to the feces, thus preventing constipation. Herbivorous animals utilize cellulose indirectly. Much of the cellulose in the food of such animals disappears in passing through the digestive tract, and it is thought that this is due to the action of microorganisms which convert cellulose into soluble products, possibly ones of use to the animal. Certain insects, notably termites, contain an enzyme **cellulase,** capable of hydrolyzing cellulose, and they can therefore utilize it as a food. The cellulase is said to be present in microorganisms in the digestive tract of termites.

Since cellulose gives glucose on hydrolysis, it would appear that a food could be prepared from it. However, it has not proved practicable to do this because of the difficulty of separating the sugar from the hydrolyzed mixture. Hydrolyzed cellulose has been employed in the fermentation industry for the production of alcohol, and some use has been made of it as a cattle feed.

Normal cellulose is insoluble in water but is soluble in an ammoniacal solution of $Cu(OH)_2$, which is called **Schweitzer's reagent.** A solution of $ZnCl_2$ in HCl, called **Cross and Bevan's reagent,** also dissolves it. When treated with H_2SO_4, it is converted into a material called **amyloid,** which gives a blue color with iodine. Further action of H_2SO_4 converts it into glucose.

INDUSTRIAL USES OF CELLULOSE. Industrially, cellulose and its derivatives are among the most important products of biological origin. In the forms of cotton and linen cloth, rope, and paper, cellulose products have been in use for a long time. More recently cellulose derivatives have come into wide use. One of the first products to attract attention was **mercerized cotton,** which is made by treating cotton goods with alkali. By this process cotton materials are made stronger, they dye better, and they have a silky appearance.

An important use of cellulose is in the manufacture of **rayon.** In making rayon, cellulose derivatives such as cellulose xanthate, known as viscose, or cellulose acetate are converted into filaments which are spun into threads. The thread is then woven into fabrics.

Cellophane is made from either viscose or cellulose acetate. In its manufacture solutions of viscose or cellulose acetate are forced through narrow slots and form sheets of transparent material. These sheets are passed through a solution of glycerol to render the cellophane more pliable and are finally coated with lacquer to make the cellophane waterproof.

Cel-O-Glass is made by coating wire screening with cellulose acetate. It is often preferable to glass because it allows the ultraviolet rays of sunlight to pass through. Cel-O-Glass has found wide use in poultry houses and for sun porches because of the health-giving properties of ultraviolet light.

Other uses of cellulose acetate are in the manufacture of shatterproof glass for automobiles and for motion-picture films. Shatterproof glass is made by cementing two layers of glass together with a layer of cellulose acetate. Since cellulose acetate is less inflammable than are the materials ordinarily used for motion-picture films, this material is used for home-movie films.

Cellulose nitrate has many important industrial uses. In cellulose nitrate the free OH groups of cellulose are replaced by NO_3. If the cellulose is almost completely nitrated, an important explosive, known as **gun cotton,** results. This is used in making smokeless powder.

A less highly nitrated cellulose known as **pyroxylin** has various applications. Dissolved in a mixture of alcohol and ether, it gives **collodion,** which is used in the laboratory to make dialysis membranes. A more familiar use is in the product known as New Skin. When New Skin is applied to a wound, the solvent evaporates, leaving a transparent protective coating over the wound. **Celluloid** is a mixture of pyroxylin and camphor. Dissolved in a suitable solvent and sprayed on cloth, pyroxylin is used to make imitation leather. Perhaps the most important commercial use of pyroxylin is in the manufacture of lacquers for finishing automobiles and many other articles.

Fructosans. INULIN. Inulin is a fructose polysaccharide composed of about 30 D-fructose units linked together by glycosidal linkages. The fructose in inulin has a furanose structure as is the case in sucrose. It may be hydrolyzed by either acid or the enzyme **inulase** directly to D-fructose. It is soluble in hot water, levorotatory, gives no color with iodine, and does not reduce Fehling's solution. Inulin is found in the roots of chicory, dahlia, dandelion, and the Jerusalem artichoke, its main source being chicory. Commercial growing of the Jerusalem artichoke as a source of fructose, which would be of value because of its sweetness, has been suggested. Inulin is of no value as a food, since there are no enzymes in the digestive tract capable of hydrolyzing it. When fed to an animal, it is excreted unchanged in the feces.

Inulin is not the only fructosan known. Several others exist which have been isolated from the roots of higher plants and found to differ from inulin in molecular size and chemical structure. One, called **levan,** is synthesized by several species of bacteria when grown on media containing sucrose or raffinose.

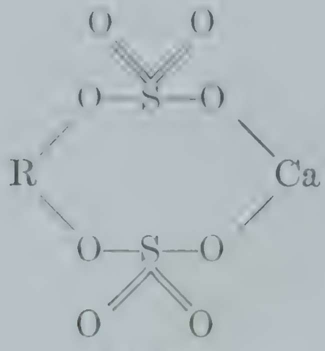
Mannosans. VEGETABLE IVORY. Vegetable ivory is a mannose polysaccharide. It is a material obtained from the ivory nut and used in the manufacture of buttons. It is a good source of the monosaccharide mannose, which may be obtained from it by hydrolysis. Other mannosans are known, notably those found in coniferous woods. The mannosans are similar to cellulose in that the mannose units are linked together by **β-1,4-glycosidic linkages.**

Mixed Polysaccharides. It will be noted that all of the polysaccharides discussed thus far give a single monosaccharide on hydrolysis. This is not the case with the mixed polysaccharides which give various pentoses, hexoses, uronic acids, and in some instances nonsugar derivatives on hydrolysis. It is evident mixed polysaccharides are complicated chemical structures. In this group we find the gums, mucilages, hemicelluloses, and compound celluloses.

Gums. The gums are complex carbohydrates which yield **pentoses, hexoses,** and **uronic acids** on hydrolysis. The uronic acids present give gums an acidic property. Gums often occur in plants in the form of metallic salts. Most gums are partially soluble in water, forming sticky colloidal solutions. The gums occur in plants not as a normal product but usually as a result of abnormal conditions, such as disease or insect bites. Common gums are gum arabic, often called gum acacia, and gum tragacanth. Injections of sterile solutions of gum arabic into the blood stream have been used to increase the blood volume after severe hemorrhages. Gum tragacanth is used as a constituent of hand lotions.

Mucilages. The mucilages are normal constituents of many plants, and one function which has been attributed to them, because of their water-holding capacity, is that they aid plants in retaining water. In hot water they form colloidal solutions which gel on cooling. Because of their water-holding ability plant mucilages have been employed in place of blood for transfusions to maintain blood volume after severe hemorrhage. Mucilages prepared from okra are said to be especially good for this purpose.

One of the best examples of a mucilage is **agar-agar,** which is obtained from a seaweed. From 20 to 28 per cent of agar-agar is composed of a polysaccharide which gives galactose on hydrolysis. The polysaccharide is combined with H_2SO_4 in ester linkage with OH groups. Only one of the acid hydrogens of H_2SO_4 is involved in the ester linkage, the other being in the form of a metallic salt. The chemical structure may be represented thus, R being the polysaccharide:



Agar-agar

If the Ca is removed, an acid known as **agar acid** results. Solutions of agar acid will not gel, but solutions of agar salts will.

Agar-agar is of special interest because of its use in the preparation of solid culture media in bacteriology. At the temperature of boiling water it forms a sol, which changes to a gel at about 40° C. Since bacteria will withstand temperatures of 40° C. for short periods of time, they may be suspended in agar-agar sols, and these sols may be poured on plates to cool and solidify. When these agar-agar plates are placed in an incubator, the bacteria dispersed throughout the gel grow and form individual colonies which may be removed and transplanted to new media in test tubes, thus giving pure cultures of the organism.

There are no enzymes in the digestive tract capable of hydrolyzing agar-agar, so that any benefits derived from it are indirect, as is true also of cellulose. Agar-agar can relieve constipation; because of its water-holding capacity, it gives bulk to the feces, making the stool softer and more easily eliminated. **Petrolagar** is a combination of mineral oil and agar-agar widely sold to relieve constipation.

Hemicelluloses. Hemicelluloses differ from normal cellulose in that they give other sugars than glucose on hydrolysis. They yield mannose, galactose, and pentoses, together with uronic acids. They are found in such materials as nutshells and stony seeds. Wood contains about 20 per cent of hemicelluloses, which are more easily hydrolyzed than normal cellulose.

Compound Cellulose. Three kinds of compound cellulose are generally recognized, namely, **lignocellulose**, **adipocellulose**, and **pectocellulose**, which, as the names imply, are combinations of normal cellulose with other compounds. That normal cellulose is actually in chemical combination with the associated compound is doubtful. Possibly the associated compound is merely an encrusting material. The various classes of compound celluloses differ with respect to the other compounds present.

Lignocellulose is composed of normal cellulose and **lignin.** It is found in woody tissue both in the cell wall and in the intercellular spaces known as the middle lamella. The chemical structure of lignin has not been determined, but we know that it contains pentosans and aromatic compounds. Among the aromatic compounds which have been isolated from lignin are vanillin and coniferyl alcohol. Lignin contains methoxy groups.

Since in making paper pulp lignin must be removed from the wood, and since wood contains about 25 per cent of lignin, it is an important by-product of the pulp industry. Several uses have been made of this by-product. From the vanillin artificial vanilla extract has been made. Lignin itself, combined with other materials, has been used in making plastics, for roofing materials, and as a binding material in road building. Used in storage battery plates, it increases the efficiency of batteries in cold weather.

Adipocellulose is normal cellulose associated with a substance called **cutin,** which has a fatty nature. It is found in corky tissue.

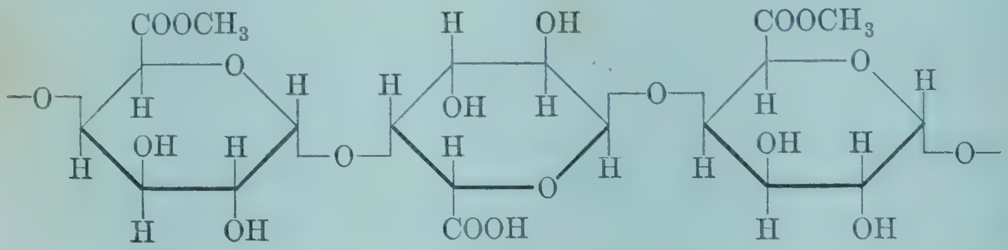
Pectocellulose is a combination of cellulose and a pectic substance called **protopectin.** Protopectin, like lignin, is found in the middle lamella and is the material which holds plant cells together. Protopectin and lignin do not occur together to any extent; when one is present, the other is usually absent. Protopectin is found in fruits, especially citrus fruits and apples, whereas lignin is found in woody tissue.

When fruits containing pectocellulose are boiled for a long time with water, the pectocellulose is hydrolyzed, and the protopectin is converted into pectin. In combination with sugar and acid this pectin forms a jelly, which is familiar in the form of fruit jellies. For jelly formation, 0.3 to 0.7 per cent pectin and 65 to 70 per cent sugar must be present, and the pH must be between 3.2 and 3.5. Commercial pectin, made from apple pumace and cull citrus fruits, and utilized in making jellies from fruits which contain little pectin, is now on the market. In addition to making jellies, pectin has other important uses. It is sometimes added to bakery products to aid in keeping them fresh. Added to fruits which are to be preserved by freezing, pectin helps to maintain firmness and color. It is also used as a constituent of skin lotions.

Medicinally pectin has been employed in several ways. Added to milk, it helps to control diarrhea in infants. It has also been used in colitis in adults. Injected into the muscle, it lowers the clotting time of blood and for this reason has been used in internal hemorrhage. Perhaps the old saying, "An apple a day keeps the doctor away," arose from the fact that apples are rich in pectin and that pectin has important therapeutic properties.

An interesting derivative of pectin which has been of value in treating such diseases as colitis is **nickel pectin.** Pectin has acidic properties and will form salts with metals. The nickel salt has high germicidal activity, and at the present time it is made commercially and is used in medicine.

The chemical structure of pectin is thought to be similar to that of starch, except that galacturonic acid molecules, rather than glucose, are the units linked together in long chains of possibly two hundred units. Since such a linkage would not involve the carboxyl groups of the galacturonic acids these should be free. However it is believed that many of the carboxyl groups are in ester formation with methyl alcohol. The following formula for a portion of the pectin molecule has been suggested:



Pectin

Note that the center unit has a free carboxyl group and that the end units exist as methyl esters.

Immuno-polysaccharides. Many bacteria, when grown on culture media, excrete into the medium soluble polysaccharides which differ in composition, depending on the organism which produces them. When these polysaccharides are injected into animals, specific antibodies appear in the blood. For this reason these polysaccharides have been called immuno-polysaccharides. These antibodies, which are also produced when an animal becomes infected with a disease organism, are thought to be important factors in the resistance to a specific disease which an animal develops after having had a disease or after having been vaccinated with suspensions of the dead organism. Not much is known concerning the exact structure of the immuno-polysaccharides, but we know something about the products which they give on hydrolysis. Among these products hexoses, pentoses, amino sugars, and uronic acids have been found.

The Determination of Carbohydrates. Most of the common methods for determining carbohydrates depend upon the reduction of copper solutions, such as Fehling's solution, and the subsequent determination of the amount of Cu_2O produced by weighing directly, by titration methods, or by colorimetric procedures. Di- and polysaccharides that do not reduce Fehling's solution, such as sucrose and starch, are first hydrolyzed by treatment with acid or enzymes. Polariscopic methods are also widely used.

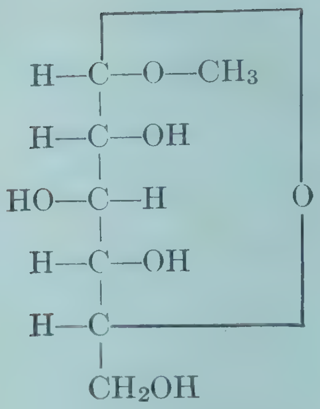
In the analysis of feeds two common methods should be familiar to the biochemist, namely, the determination of **crude fiber** and **nitrogen-free extract.** In the determination of crude fiber the sample is dried and extracted with ether to remove the fat. The sample is then boiled with 1.25 per cent H_2SO_4 for 30 minutes and then with 1.25 per cent NaOH solution for the same length of time. The residue is filtered, washed, dried, and weighed, then ignited and weighed again. The loss in weight on ignition represents the crude fiber. Crude fiber consists largely of cellulose but contains some hemicelluloses and nitrogenous substances. Roughly, it represents the indigestible carbohydrate present in a feed.

Nitrogen-free extract is determined not directly but by difference. In a complete feed analysis the percentages of water, ash, protein, fat, and crude fiber are determined. The sum of these, subtracted from 100, gives the percentage of nitrogen-free extract. The nitrogen-free extract is made up largely of sugars, starch, and hemicelluloses. Roughly, it indicates the amount of digestible carbohydrates in a feed.

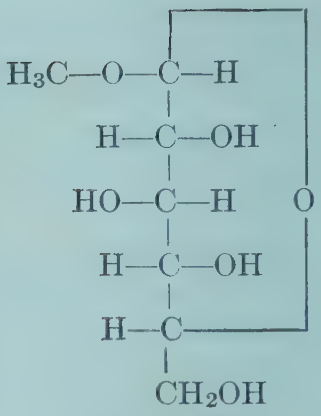
Glycosides

It will be recalled that in the consideration of the structure of di- and polysaccharides they were considered derivatives of glucose or some other sugar taken as a nucleus and were called glycosides. Although di- and polysaccharides may be considered glycosides, it is more usual to reserve the term glycoside for combinations of glucose or some other sugar with some nonsugar group.

Most glycosides are derivatives of D-glucose. In this sugar, it will be recalled, there is a 1,5-oxygen ring with an OH group on the potential aldehyde carbon atom. In glycosides the H of this OH is replaced by some group often referred to as the **aglycone group.** The simplest glycoside would be methylglucoside, in which a CH_3 group replaces the H of the OH. Since D-glucose exists in the α and β forms, it is evident that there may be two kinds of glycosides, namely, alpha and beta. All naturally occurring glycosides, according to Gortner, are of the β variety. The following formulas illustrate the structure of the two methylglucosides:



α-Methylglucoside



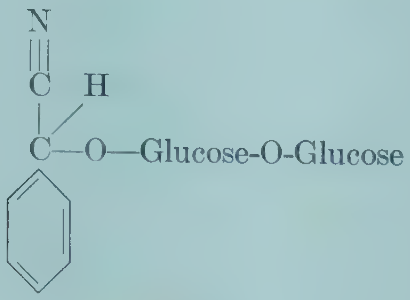
β-Methylglucoside

As has been mentioned before, the two varieties of glycosides may be distinguished by the fact that α-glycosides are hydrolyzed by the enzyme maltase, whereas β-glycosides are hydrolyzed by emulsin.

Although glucose is the common sugar found in glycosides, others may replace it. Galactose, mannose, and fructose, as well as pentoses, have been shown to be the sugar in certain glycosides.

The glycosides found in nature are much more complex than the methylglucosides just referred to. Frequently the aglycone group tied to the sugar is very complex. Often it is a compound with marked physiological properties. The medicinal properties of many plants are due to glycosides which are present. Possibly glycosides are formed in plants as a means of self-protection. Substances which might be injurious to the plant may be made inactive by combination with glucose, or the toxic constituent of the glycoside may inhibit the growth of disease-producing microorganisms or may repel insects and larger animals which might otherwise destroy or injure the plant.

Only a few glycosides will be mentioned. **Amygdalin,** which is found in bitter almonds and in peach pits, gives two molecules of glucose, benzaldehyde, and HCN on hydrolysis. The following formula represents its structure:



Amygdalin

Dhurrin, another cyanogenetic glycoside, is found in millet and sorghum. It gives *p*-hydroxybenzaldehyde, HCN, and glucose on hydrolysis. It is found especially in young plants, and many cattle have died from HCN poisoning when allowed to pasture on young millet.

Phlorhizin, found in the bark of the apple, pear, plum, and cherry, gives glucose and an aromatic compound called phloretin on hydrolysis. This glycoside is of interest because it is utilized to produce an artificial diabetes or glycosuria. **Gaultherin** yields glucose and methyl salicylate on hydrolysis. Methyl salicylate is commonly called oil of wintergreen.

Salicin is obtained from willow bark and gives glucose and salicyl alcohol on hydrolysis. On oxidation salicyl alcohol gives salicylic acid, which in the form of its salts is widely used for the relief of pain. Aspirin, which is acetyl salicylate, is perhaps the best known of these derivatives. Salicin itself is often used to relieve pain and is said to have the advantage of being more readily tolerated. **Indican** is found in the indigo plant. It gives glucose and indoxyl on hydrolysis. Until recently indican was the source of the blue dye, indigo. In the leaves and seeds of the foxglove (*Digitalis purpurea*) the **digitalis glycosides** are found. They are used in medicine as heart stimulants and yield a variety of products on hydrolysis.

REVIEW QUESTIONS

1. What is the biological importance of carbohydrates?
2. Define simple carbohydrate, aldose, ketose, monosaccharide, disaccharide, polysaccharide, pentose, and hexose.
3. Classify carbohydrates.
4. Why is it possible to have only one diose sugar?
5. Name three triose sugars.
6. If the graphic formula for a sugar is known, how may the number of optical isomers which are theoretically possible be determined?
7. What is meant by a desoxy sugar?
8. Name the four pentose sugars found in nature.
9. Write an equation showing what happens when a pentose is boiled with HCl.
10. Write graphic formulas for the four important hexoses and indicate the polyhydric alcohols from which each may be derived.
11. Describe Molisch's test. What is it a test for?
12. What is the action of alkali on reducing sugars and on nonreducing sugars?
13. What is the Lobry de Bruyn transformation?
14. What is the action of acid on mono-, di-, and polysaccharides?
15. What is a uronic acid?
16. What is meant by protective synthesis?
17. How are glucuronic, gluconic, and saccharic acids related chemically to D-glucose?

18. Give the composition of Fehling's solution and indicate the function of each constituent.

19. How does Benedict's qualitative reagent differ from Fehling's solution?

20. How does Benedict's quantitative reagent differ from his qualitative reagent? How is the quantitative reagent used?

21. What is the composition of Barfoed's solution? Of what special value is it?

22. How does Nylander's solution differ from Fehling's solution?

23. What precipitate results when reducing sugars are heated with Fehling's, Benedict's qualitative and quantitative reagents, Barfoed's, and Nylander's solutions?

24. Name several other reagents which sugar solutions will reduce.

25. Name the alcohols which the four important hexose monosaccharides give on reduction.

26. What is meant by anaerobic or intermolecular respiration?

27. Indicate the reactions taking place in the formation of an osazone from D-glucose.

28. Indicate how glucosazone may be converted into D-fructose.

29. Name an important property of the hydrazone of D-mannose which distinguishes it from the hydrazone of D-glucose.

30. Of what value are osazones?

31. Which of the common hexose monosaccharides give the same osazone? Why?

32. How do sugars react with phosphoric acid?

33. Write an equation showing what happens during the alcoholic fermentation of glucose. Name several other types of fermentation.

34. What is Schiff's test? Do simple sugars give a positive Schiff test? Why?

35. Explain mutarotation, using D-glucose as an example.

36. How may α- and ß-D-glucose be prepared?

37. Write the pyranose and furanose formulas for an aldohexose.

38. Write the Haworth formulas for α- and ß-D-glucose.

39. How do plants and animals differ in their nutrition?

40. Define photosynthesis. Give a theory explaining what happens during photosynthesis.

41. Discuss the occurrence, physiological importance, uses, and commercial manufacture of D-glucose.

42. What is glucosamine? Where is it found in nature?

43. What is the most characteristic thing about the chemical structure of D-fructose?

44. What effect does temperature have on the specific rotation of D-fructose?

45. How may D-fructose be prepared?

46. Name two tests for fructose.

47. What are the relative sweetnesses of the common sugars?

48. Give the occurrence of D-mannose and two methods of preparing it.

49. Compare the osazones and hydrazones of D-mannose and D-glucose.

50. Describe the taste of D-mannose.

51. Where is D-galactose found in nature?

52. Give two tests for galactose.

53. Into what two classes are disaccharides divided?

54. What do maltose, cellobiose, lactose, and sucrose give on hydrolysis?

55. Write the graphic and Haworth formulas for maltose, cellobiose, lactose, and sucrose, and give the chemical name for each.

56. What is a glycoside? How may α- and β-glycosides be distinguished?

57. What is malt?

58. What is the difference in chemical structure between maltose and cellobiose? From what is cellobiose obtained?

59. Give two advantages of the fact that lactose is the sugar of milk.

60. What is β-lactose? How is it made from the α-variety? What is "sandy" ice cream?

61. What are the chief sources of sucrose? Give two other names for sucrose

62. What are barley sugar and caramel?

63. Does sucrose reduce Fehling's solution, show mutarotation, or form osazones? Why?

64. Why does sucrose crystallize so readily? How may crystallization be prevented?

65. What is meant by the inversion of cane sugar? How is it done, and why is the process called inversion?

66. What is viscogen? For what is it used?

67. Indicate three ways of hydrolyzing raffinose. What products are formed with each method?

68. Where are pentosans found in nature?

69. How may pentosans be used by the body? What compounds in the body contain pentoses?

70. Where does starch occur in nature?

71. How can the origin of starch be determined?

72. What is the chemical structure of starch?

73. Name two chemical components of starch. Name the enzymes involved in the hydrolysis of starch to maltose and tell what each does.

74. How may starch be synthesized in the laboratory?

75. Give a test for starch. What is the achromatic point?

76. Give several uses of starch.

77. Name three varieties of dextrin and give their reaction with iodine.

78. How is dextrin made?

79. What is the solubility of dextrin? Does it reduce Fehling's solution?

80. What is Dextri-maltose? For what is it used?

81. Give several uses of dextrin.

82. Give the occurrence of glycogen.

83. How does the chemical structure of glycogen compare with that of starch?

84. What color does glycogen give with iodine?

85. Does glycogen reduce Fehling's solution or form osazones?

86. How is glycogen prepared?

87. How does the chemical structure of cellulose differ from that of starch?

88. Name two reagents which dissolve cellulose and give the composition of each.

89. Of what value is cellulose in the diet?

90. What is mercerized cotton?

91. What is rayon, and how is it made?

92. What is cellophane?

93. What is Cel-O-Glass? Why is it used?

94. How is shatter-proof glass made?

95. What are gun cotton, pyroxylin, collodion, celluloid, New Skin, and pyroxylin lacquer?

96. What is inulin? Where is it found, and of what value is it?

97. What is the chemical composition of gums? Where are they found? For what are they used?

98 What are mucilages, and of what value are they to plants?

99. What is agar-agar? Give its uses in bacteriology and in medicine. Is it of value as a food?

100. How do hemicelluloses differ in composition from normal cellulose?

101. Name and characterize three types of compound cellulose.

102. Give several uses of lignin.

103. What is pectin? What is its chemical structure? How is it prepared?

104. What conditions are necessary for pectin to form a jelly?

105. Give the uses of pectin.

106. What is nickel pectin, and for what is it used?

107. What are immuno-polysaccharides?

108. Indicate several methods for determining the amount of carbohydrate in a food.

109. What is meant by the terms crude fiber and nitrogen-free extract?

110. Give two possible physiological functions of glycosides in plants.

111. What is meant by an aglycone group?

112. Name several glycosides, and tell what each yields on hydrolysis.

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4

Lipids

The lipids are important constituents of protoplasm, characterized by being insoluble in water but soluble in ether, chloroform, and other fat solvents. Chemically they are esters of fatty acids or are capable of forming esters. They all contain carbon, hydrogen, and oxygen, and some have also phosphorus and nitrogen. They are found in all plant and animal matter, and at least some of them appear to be an essential constituent of protoplasm. In the animal body they form the main store of reserve food supply, being derived from the fat and carbohydrate of the diet. The brain and nervous tissue is rich in certain lipids, a fact which indicates the importance of these compounds to life.

Classification of Lipids

I. **Simple lipids** — esters of fatty acids and alcohols.
    1. Fats and oils — esters of fatty acids and glycerol. Fats are solids, whereas oils are liquids at room temperature.
    2. Waxes — esters of long-chain fatty acids and long-chain monohydric alcohols or sterols.

II. **Compound lipids** — esters of fatty acids and alcohols in combination with other compounds.
    1. Phospholipids — fatlike compounds containing phosphoric acid and a nitrogen base.
    2. Glycolipids — compounds containing a fatty acid, a carbohydrate, a complex alcohol, and nitrogen, but no phosphorus.

III. **Derived lipids** — the simple compounds that simple and compound lipids give on hydrolysis.
    1. Fatty acids.
    2. Alcohols.
    3. Sterols.

Before the fats and oils, the first group in the above classification, are discussed, it will be necessary to consider some of the derived lipids which are components of these compounds. Since all fats and oils are esters of fatty acids and glycerol, these compounds should be considered before continuing our discussion of the fats and oils.

Fatty Acids, Soaps, and Glycerol

The Fatty Acids. The fatty acids are important because they are found in all the simple and compound lipids. Most of the fatty acids found in the lipids are straight-chain acids, although branched-chain acids have been found in certain fats. Many of the important acids are unsaturated and occur in both fats and oils but are especially abundant in oils. A few acids, both saturated and unsaturated, contain hydroxyl groups and some are cyclic in structure.

TABLE 5

THE FATTY ACIDS FOUND IN LIPIDS

Name	Formula	Occurrence
I. Saturated Acids		
1. Butyric	C_3H_7COOH	Butter
2. Caproic	$C_5H_{11}COOH$	Butter
3. Caprylic	$C_7H_{15}COOH$	Coconut
4. Capric	$C_9H_{19}COOH$	Palm nut
5. Lauric	$C_{11}H_{23}COOH$	Laurel and coconut oils
6. Myristic	$C_{13}H_{27}COOH$	Nutmeg and mace
7. Palmitic	$C_{15}H_{31}COOH$	Palm oil and lard
8. Stearic	$C_{17}H_{35}COOH$	Tallow
9. Arachidic	$C_{19}H_{39}COOH$	Peanut
10. Behenic	$C_{21}H_{43}COOH$	Ben oil
11. Lignoceric	$C_{23}H_{47}COOH$	Peanut
12. Cerotic	$C_{25}H_{51}COOH$	Waxes
13. Melissic	$C_{29}H_{59}COOH$	
II. Unsaturated Acids		
1. Oleic	$C_{17}H_{33}COOH$ 1 =	Olive oil
2. Linoleic	$C_{17}H_{31}COOH$ 2 =	Corn oil
3. Linolenic	$C_{17}H_{29}COOH$ 3 =	Linseed oil
4. Arachidonic	$C_{19}H_{31}COOH$ 4 =	Lecithin and cephalin
5. Clupanodonic	$C_{21}H_{33}COOH$ 5 =	Fish oils
III. Hydroxy Acids		
1. Ricinoleic	$C_{17}H_{32}(OH)COOH$	Castor oil
2. Cerebronic	$C_{23}H_{46}(OH)COOH$	Phrenosin
3. Dihydroxystearic	$C_{17}H_{33}(OH)_2COOH$	Castor oil
IV. Cyclic Acids		
1. Hydnocarpic	$C_{15}H_{27}COOH$	Chaulmoogra oil
2. Chaulmoogric	$C_{17}H_{31}COOH$	Chaulmoogra oil

A rather important generality which may be pointed out in connection with the fatty acids mentioned in Table 5, which are the important ones found in nature, is that they all contain an even number of carbon atoms. Although fatty acids with an odd number of carbon atoms have been found in fats, their occurrence is rare. The saturated acids in the series up to and including **capric acid** are volatile when they are subjected to steam distillation. The longer-chained fatty acids are solid at 20°C. and are nonvolatile. This volatility of the lower fatty acids is utilized in testing for butterfat, which is characterized by its high percentage of volatile fatty acids. **Palmitic** and **stearic acids** are the most important of the saturated fatty acids. They are found in most of the common fats and oils. The saturated fatty acids with longer carbon chains are of interest because they are found in many of the common waxes.

The unsaturated fatty acids occur as glycerides especially in oils. The oils are liquids, largely because of the unsaturated fatty acids present. The most important unsaturated fatty acids are **oleic** $[CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH]$, **linoleic** $[CH_3(CH_2)_4CH{=}CH\text{-}CH_2CH{=}CH(CH_2)_7COOH]$, and **linolenic** acids $[CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH]$. It will be noticed that all three have double bonds in the molecule and that unsaturation progresses from oleic acid, with one double bond, to linolenic acid, with three.

Certain unsaturated fatty acids are indispensable in the diet. If rats are fed a fat-free diet they fail to grow and their skin becomes scaly. These conditions do not develop if linoleic, linolenic, or arachidonic acid is added to their diet.

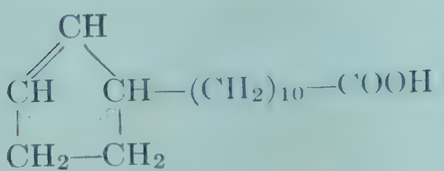
The unsaturated fatty acids present in drying oils are very important in the drying of paints. Oxidation at the double bonds of the acids is the initial step in this process. Fish oils, which have a high percentage of unsaturated fatty acids, are often used in cheap paints because of their ability to dry.

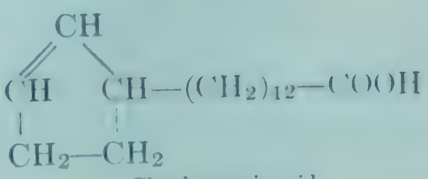
The hydroxy acids, **ricinoleic** and **dihydroxystearic,** are found in castor oil. Ricinoleic acid has the following formula:

$$CH_3(CH_2)_5CHOHCH_2CH{=}CH(CH_2)_7COOH$$

Salts of this acid have been used in colitis to detoxify the intestine. Castor oil, used medicinally, may have a dual action, namely, as a laxative and as a detoxifying agent. The laxative action of castor oil is said to be due, at least in part, to the ricinoleic acid present.

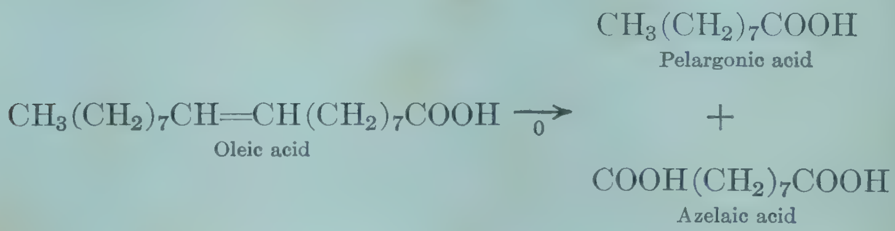
The cyclic acids, **hydnocarpic** and **chaulmoogric,** are found in chaulmoogra oil, which is of interest because it has been used in the


Hydnocarpic acid


Chaulmoogric acid

treatment of leprosy. At the present time the ethyl ester of chaulmoogric acid is used instead of the oil. Hydnocarpic and chaulmoogric acids, besides being cyclic in structure, are also unsaturated as each contains one double bond.

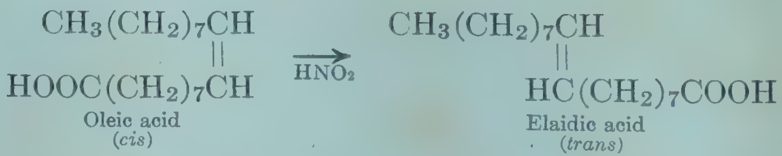
Isomerism in Fatty Acids. Three types of isomerism may occur in fatty acids. Oleic acid has one double bond, which is located in the center of the molecule. On oxidation oleic acid breaks down into one molecule of **pelargonic acid** and one of **azelaic acid.**



$$\underset{\text{Oleic acid}}{CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH} \xrightarrow[O]{} \begin{array}{c} \underset{\text{Pelargonic acid}}{CH_3(CH_2)_7COOH} \\ + \\ \underset{\text{Azelaic acid}}{COOH(CH_2)_7COOH} \end{array}$$

If the cleavage is assumed to have taken place at the double bond, its position must have been in the center of the molecule, because both pelargonic and azelaic acids have nine carbon atoms. As the position of the double bond in oleic acid may appear in sixteen places, sixteen isomers are possible. Several of these isomers are known. This type of isomerism is known as **position isomerism.**

Another type of isomerism which occurs in unsaturated fatty acids is known as **geometric isomerism.** This depends on the spatial arrangement of groups tied to the double-bonded carbon atoms. If oleic acid, which is liquid, is treated with nitrous acid, it is converted into a solid called elaidic acid. Both oleic and elaidic acids have the same empirical formula but differ in the arrangement of groups in relation to the double bond.



$$\begin{array}{r} CH_3(CH_2)_7CH \\ \| \\ HOOC(CH_2)_7CH \\ \text{Oleic acid} \\ (\textit{cis}) \end{array} \xrightarrow[HNO_2]{} \begin{array}{l} CH_3(CH_2)_7CH \\ \quad\quad\quad\quad\| \\ \quad\quad\quad\quad HC(CH_2)_7COOH \\ \quad\quad\quad\quad\text{Elaidic acid} \\ \quad\quad\quad\quad(\textit{trans}) \end{array}$$

In oleic acid both hydrogens tied to the double-bonded carbons are represented as being on the same side of the molecule; therefore oleic acid is spoken of as the ***cis* form.** In elaidic acid these hydrogens are represented as being on opposite sides of the molecule; therefore elaidic acid is spoken of as the ***trans* form.** Geometric isomerism is sometimes referred to as ***cis-trans* isomerism.**

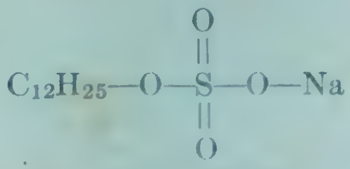
It should also be pointed out that **optical isomerism** may occur in fatty acids containing asymmetric carbon atoms. In ricinoleic acid and other hydroxy acids the carbon to which the hydroxyl group is attached is asymmetric, and therefore these acids are optically active and may exist in either the D or the L form.

Since both hydnocarpic and chaulmoogric acids contain an asymmetric carbon atom, they also are optically active.

Soaps. The metallic salts of the fatty acids are called **soaps.** Sodium and potassium salts are soluble in water and are the common soaps in the home. The salts of calcium and magnesium and the heavy metals, such as lead and zinc, are insoluble in water. Sodium salts are known as **hard soaps** and potassium salts as **soft soaps.** When soap is dissolved in hard water, it reacts with the calcium and magnesium in the water to form insoluble soaps, which precipitate, leaving the water soft. **Zinc soaps,** such as zinc stearate, are often used as dusting powder for babies, because their action is antiseptic and because they do not wet as other powders do. Since zinc stearate is poisonous, care should be taken to prevent a baby from eating it.

The value of soaps in washing depends upon their property of lowering surface tension, which in turn aids in emulsification. Most dirt is held to the skin or to clothes by oily substances present. With soap and rubbing, this oil is emulsified and rinsed off, carrying the dirt along with it.

A very interesting product is now being used in large quantities as a substitute for soap. Commercially it is known as **Gardinol**, but to the housewife it is familiar as Dreft and Drene. Gardinol is a mixture of the sodium salts of the sulfuric acid esters of long-chain alcohols, such as lauryl or cetyl alcohol. These alcohols are prepared by reducing the corresponding long-chain fatty acids. The formula for the lauryl derivative is:

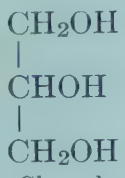


$$C_{12}H_{25}—O—\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O}{\|}}{S}}—O—Na$$

Sodium lauryl sulfate

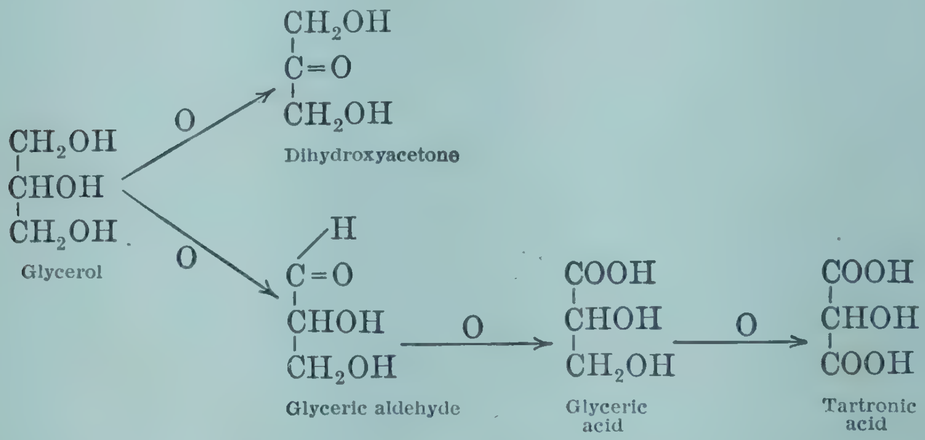
The main advantage of Gardinol over a soap is that it does not form insoluble compounds with calcium and magnesium and therefore may be used in hard water. Another advantage is that a solution of Gardinol is not alkaline, as are solutions of soaps, and therefore it may be used to wash delicate fabrics which might be injured by soap. Gardinol is more surface-tension active than soaps; and, since none is used for softening the water, much smaller quantities are needed than of soap.

Glycerol. The common constituent of all fats and oil is **glycerol.** Glycerol is a trihydric alcohol and therefore shows all the chemical reactions of alcohols. Some of the compounds formed on oxidation are represented in the following diagram:

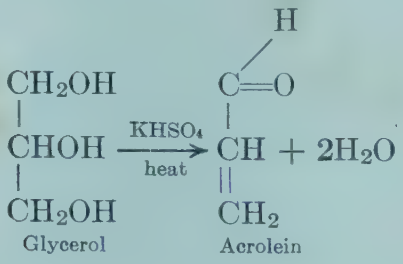


$$\begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

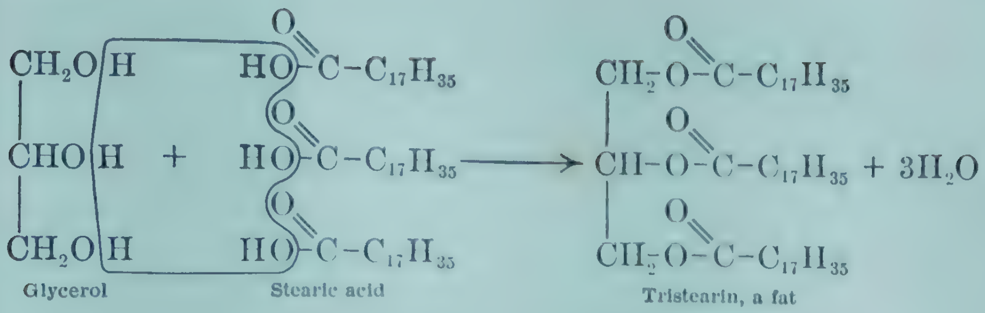
Glycerol


O
O
Glycerol
Dihydroxyacetone
Glyceric aldehyde
O
Glyceric acid
O
Tartronic acid

An interesting reaction takes place when glycerol is heated to a high temperature or with a dehydrating agent at a lower temperature. It loses two molecules of H_2O, forming **acrolein,** a compound with a very pungent odor. When beefsteak is fried on a very hot pan, acrolein may be formed through the decomposition of the glycerol in the fat. In making tallow candles, free fatty acids are often used rather than fat. When candles made from fat are burned, they produce a bad odor, due to the formation of acrolein from the glycerol present.


KHSO₄
heat
Glycerol
Acrolein

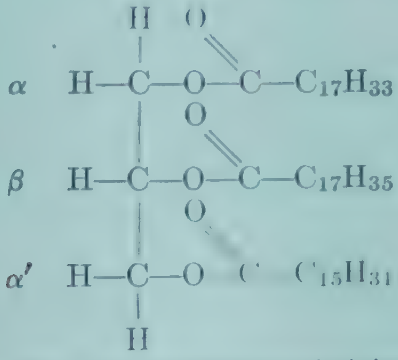
Since glycerol is a trihydric alcohol, it will form triple esters with fatty acids. Such esters are the fats and oils. The formation of a fat may be represented thus:



Fats and Oils

The fats and oils are very similar in their chemical structures, both being esters of glycerol and fatty acids. If the glyceride is a solid at room temperature, it is classed as a fat; if it is a liquid at that temperature, it is classed as an oil. Although there is no sharp chemical difference between fats and oils, it should be pointed out that the presence of long-chain, saturated fatty acids in a glyceride is associated with a high melting point, whereas short-chain and unsaturated fatty acids are associated with a low melting point.

Chemical Structure. In the preceding section the formula for **tristearin,** a typical fat, was given. When all the fatty acids in a fat molecule are the same, as they are in tristearin, the fat is called a **simple glyceride.** In most naturally occurring fats different fatty acids are found in the same fat molecule. Such a fat molecule is called a **mixed glyceride.** It is evident from the following formula that several isomers of a mixed glyceride may exist.



α-Oleo-α′-palmito-β-stearin (a mixed glyceride)

It is possible to have glycerides with only one or two fatty acids in the molecule, and such molecules occur in nature.

Animal Fats. Most of the glycerides found in animals are fats, rather than oils, although in many of the cold-blooded animals, such as fish, oils are found. The fats of animals of different species are often quite different in composition. Since the melting point of a fat varies with its chemical composition, it may be used to show differences in composition. Lard, which is hog fat, has a melting point of 28°C., beef tallow 46°C., and sheep fat 51°C. Thus it can be seen that hogs have shorter-chained or more unsaturated fatty acids in their fat than cows or sheep.

Fats from different parts of the body of the same animal vary in composition. Thus hog kidney fat melts at 43°C., whereas lard, which is the body fat of the hog, melts at 28°C. As a rule the fats in the more active parts of the body are more unsaturated and have lower melting points than those stored in fatty tissue for future use. The fats in the heart muscle are highly unsaturated. Unsaturated fats are more easily oxidized than saturated ones, a fact which may account for the prominence of the unsaturated fats in tissues where metabolism is active.

Diet also influences the composition of body fat. Up to a certain point the body is able to deposit a fat of rather definite composition; but, when large quantities of fat are fed, the body tends to deposit a fat similar in composition to that in the diet. Perhaps the best example is found in the hogs raised in the South. When large quantities of peanuts or cottonseed meal are fed them, the fat becomes soft and the hogs are not so marketable.

Butterfat is a very important animal fat. The chief characteristic which distinguishes it from other fats is its high percentage of volatile soluble fatty acids. From 10 to 13.6 per cent of the fatty acids in butterfat are of this type. Adulteration of butter can easily be recognized by determining its volatile soluble fatty acid content.

Oleomargarine. Many substitutes for butter are on the market at the present time. The first substitute was made in 1870 and was called oleomargarine. It was made from beef fat by partly melting and separating the more liquid fraction. This oleo oil was mixed with other fats and oils, churned with milk, and worked up like butter. At the present time many products of this kind are on the market. Many contain large quantities of such low-melting glycerides as those of coconut oil.

A great deal of the prejudice against the use of oleomargarine or artificial butters is unwarranted. From the standpoint of purity they should be better than many butters, and as foods they possess the same energy values as butter. One argument in favor of butter is the fact that butter is a good source of vitamin A, whereas many butter substitutes are not. At the present time many butter substitutes are being fortified by the addition of vitamin A. Another argument in favor of butter has been its superior spreading qualities. This superiority has been overcome in many butter substitutes by careful selection of the fats and oils used in their preparation.

Vegetable Oils. Most of the glycerides found in the vegetable kingdom are oils rather than fats. That they are oils is attributed mainly to the presence of unsaturated fatty acids in the molecule. Since unsaturation is an essential for drying, many of the vegetable oils are drying oils. In fact, the vegetable oils are classified on the basis of their drying properties.

Classification of Vegetable Oils

1. **Nondrying oils,** containing large quantities of oleic acid.

Palm oil.	Peanut oil.
Coconut oil.	Date oil.
Olive oil.	Rice oil.

2. **Semidrying oils,** containing mainly oleic and linoleic acids.

Corn oil.	Brazil nut oil.
Cottonseed oil.	Soybean oil.
Wheat oil.	Rape seed oil.
Sesame oil.	

3. **Drying oils,** containing mainly linoleic and linolenic acids.

Linseed oil.	Hempseed oil.
Tung oil.	Walnut oil.
Poppyseed oil.	Sunflower oil.

Hydrogenation of Oils. Although vegetable oils have many uses as such, many oils, such as cottonseed oil, have been made more valuable by being converted into fats, which are used as lard substitutes. In this conversion of an oil into a fat a very simple principle of organic chemistry, namely, that hydrogen may be added to an unsaturated molecule, converting it into a saturated one, is applied. This process is called **hydrogenation.** In hydrogenation an oil is mixed with finely divided nickel, which acts as a catalyst, placed in a tank, where hydrogen may be mixed with it under pressure, and the mixture heated. By controlling the amount of hydrogen, any desired degree of hydrogenation may be obtained. The resulting mixture is then filtered to remove the nickel, and on cooling a fat is obtained. By this process cottonseed, peanut, and other oils are converted into valuable lard substitutes, such as Crisco, Snowdrift, and Spry.

Drying Oils. Perhaps the most valuable vegetable oils are those that have the property of combining with the oxygen of the air and changing to resinous solids. That oils combine with oxygen when they dry is shown by the fact that they increase in weight during the drying process. The drying process not only is an **oxidation** but also includes a **polymerization** in which many of the oxidized oil molecules unite to form a complex resinous material. The drying oils find their greatest utility in the manufacture of paints and varnishes. The most common ones are **linseed** and **tung oils.** Linseed oil is made from flaxseed either by squeezing out the oil with pressure (the **old process**) or by extracting the pressed cake with fat solvents (the **new process**). The material left after the removal of the oil is known as linseed cake and is used as a cattle feed. It should be pointed out that old-process cake, which contains much oil, is more valuable as a feed than the new-process cake.

Two kinds of linseed oil are on the market, raw and boiled. Raw linseed oil is the oil as it is extracted from flaxseed. Boiled linseed oil is prepared by heating the raw oil to 130°C. with a mixture of lead oxide and manganese dioxide. These oxidizing agents initiate the drying process; therefore boiled linseed oil dries much faster than the raw oil.

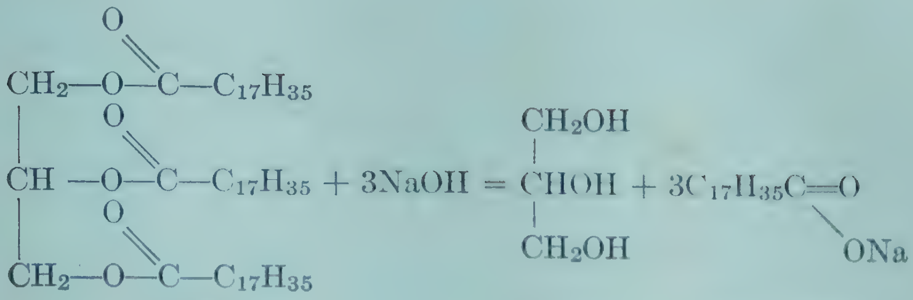
Linseed oil is often used as a laxative in veterinary medicine. It is important that the raw oil be used for this purpose since boiled oil contains lead and is poisonous.

In the drying of an oil, heat is generated. It frequently happens that oily rags generate enough heat to catch fire. Many disastrous fires have been caused by the **spontaneous combustion** of oily rags.

Hydrolysis of Fats and Oils. One of the most important reactions of fats is hydrolysis. During hydrolysis a fat is broken down into glycerol and fatty acids. This reaction may be brought about in several ways. Water alone at ordinary temperatures will hydrolyze fats in extremely long periods of time. With superheated steam the reaction will take place in a few hours.

By means of chemical reagents the hydrolysis of a fat proceeds more rapidly. A solution known as **Twitchell's reagent** hydrolyzes fats rapidly when the mixture is heated with steam at 100°C. This reagent is made of naphthalene, oleic acid, and sulfuric acid. When small amounts of it are added to a mixture of fat and water and the mixture is heated to the boiling point, the fat hydrolyzes to glycerol and free fatty acids. The Twitchell process is used extensively in soap making at the present time. The free fatty acids are treated with alkali to form soap. The main advantages in this process are that the fatty acids are easily separated from the mixture, and the residue is a water solution of nearly pure glycerol. The glycerol is a valuable by-product, and it is important that it may be easily recovered.

The most common method of hydrolyzing fats in the laboratory is by heating in the presence of alkali. If the alkali is used in alcoholic solution, the reaction is more rapid than if it is used in water solution. The reason is that the fats are slightly soluble in hot alcohol and insoluble in water. In this reaction glycerol and soap are formed thus:



Tristearin Glycerol Sodium stearate, a soap

When soaps are made by this process, they are in solution mixed with the glycerol and the NaOH. They are separated by salting out with NaCl.

From the biological standpoint the enzymic hydrolysis of fats is of interest. A fat-splitting enzyme is called a **lipase.** It splits a fat into glycerol and free fatty acids. The most important lipase of the digestive system is **pancreatic lipase** or **steapsin,** found in the pancreatic juice.

Rancidity. Most fats and oils, with age, develop a bad odor and taste and are said to become rancid. At the present time two types of rancidity are recognized, namely, **hydrolytic** and **oxidative.**

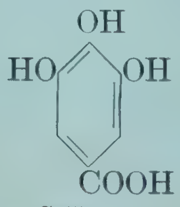
In hydrolytic rancidity the bad odor and taste are due to the free fatty acids liberated when the fat is hydrolyzed. In a fat like butterfat, which contains a high percentage of volatile fatty acids, hydrolytic rancidity is very noticeable, because the volatile fatty acids liberated have an extremely disagreeable odor and taste. On the other hand, in a fat like lard, which contains few volatile fatty acids, hydrolytic rancidity is not such a serious problem.

In most fats oxidative rancidity is the more important. This type of rancidity is caused by the oxidation of unsaturated fatty acids at their double bonds with the production of shorter-chained acids, aldehydes, and ketones. In the dairy industry **tallowy butter** is a result of oxidative rancidity. Oxygen is necessary for oxidative rancidity to occur. Light, heat, moisture, and traces of certain metals, such as copper, hasten the oxidative process. Oxidative rancidity is of great importance in cooking and baking when the products are to be kept for any length of time. Staleness in food products like potato chips and crackers is a result of oxidative rancidity. Products kept away from air and light in sealed containers remain fresh much longer than those stored in the open. Potato chips are often marketed in sealed, colored Cellophane bags to exclude air and injurious light rays which would hasten the development of rancidity.

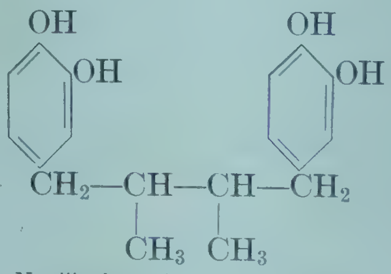
As fats vary in their ability to withstand oxidative rancidity, in the baking industry it is important to choose a fat with good keeping qualities. The keeping quality of a fat may be determined by exposing a sample to oxygen and noting the time required for active oxidation to start as indicated by an increase in weight. A fat of poor quality will start to oxidize almost immediately, whereas one of good keeping quality requires several hours for rapid oxidation to begin. The time required for the onset of rapid oxidation is known as the **induction period.**

The longer induction period of some fats may be due to the presence of traces of substances called **antioxidants,** which prevent the oxidation of double bonds. Not a great deal is known about the chemical nature of the naturally occurring antioxidants, but in general it may be said that they are compounds which are more easily oxidized than the fats that they protect from oxidation. Certain vitamins, such as C and E, are natural antioxidants.

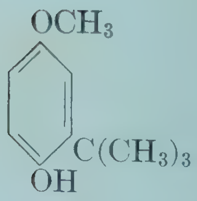
Many artificial antioxidants are known, however, a number of these cannot be added to edible fats and oils because of their toxic properties. Among those that have found wide usage are gallic acid and its esters, nordihydroguaiaretic acid (NDGA), 3-tertiary butyl-4-hydroxy anisole, and dilaurylthiodipropionate. 3-Tertiary butyl-4-hydroxy anisole is added to fats used in making potato chips because it withstands high temperatures.



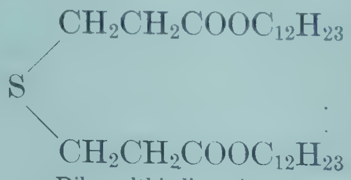
Gallic acid



Nordihydroguaiaretic acid (NDGA)

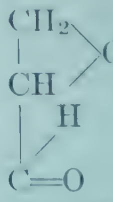


3-Tertiary butyl-4-hydroxy anisole



Dilaurylthiodipropionate

A delicate test for oxidative rancidity which is commonly used is the **Kreis test.** In this test the fat to be examined is treated with ether, phloroglucinol, and hydrochloric acid; a positive result is indicated by a red color. This test is due to the presence of **epihydrine aldehyde,** which is one of the products of fat oxidation.

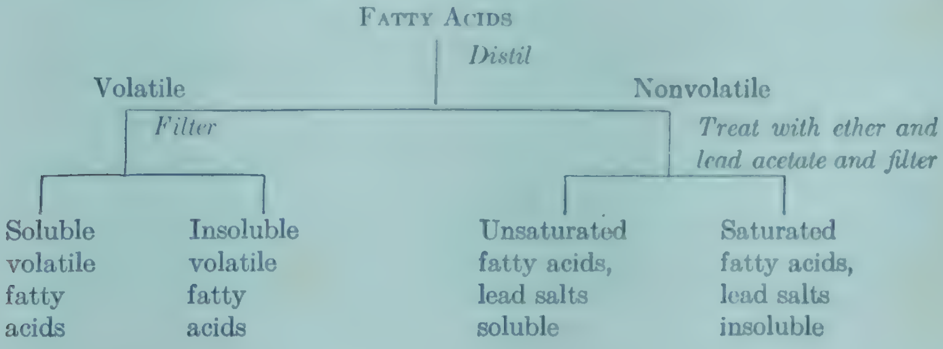


Epihydrine aldehyde

The Analysis of Fats. An ideal analysis of a fat would be the determination of the kinds and amounts of the fatty acids present, together with their relative positions in the fat molecule. Even after the amount of each fatty acid present is known, it is very difficult to tell in which positions the acids are tied to glycerol, because most fats are mixed glycerides. Since fatty acids which differ by only a few carbon atoms in the chain have such similar properties, it is a very difficult process to separate them quantitatively. It is rather simple to separate the fatty acids into large groups but very difficult to separate the individual fatty acids. If a fat is saponified with alkali and then H_2SO_4 is added, the fatty acids are set free. By a process of distillation the volatile fatty acids may be separated from the nonvolatile. Since some of the volatile fatty acids are insoluble in water, they will crystallize out in the distillate, and by filtration the volatile fatty acids may be separated into two groups, namely, the soluble and the insoluble. The volatile fatty acids include those with ten or less than ten carbon atoms in the molecule.

The nonvolatile fatty acids in the residue after distillation consist of the longer-chained fatty acids, both saturated and unsaturated. The saturated acids may be separated from the unsaturated by taking advantage of the fact that the lead salts of the unsaturated acids are soluble in ethyl ether.

The diagram presented here indicates the means of separation of the fatty acids into the four groups.


FATTY ACIDS
Distil
Volatile
Nonvolatile
Filter
Treat with ether and lead acetate and filter
Soluble volatile fatty acids
Insoluble volatile fatty acids
Unsaturated fatty acids, lead salts soluble
Saturated fatty acids, lead salts insoluble

The usual method for studying fats and oils does not include a complete analysis but rather the determination of **fat constants.** (See Table 6.) By determining several of these constants, it is usually possible to decide what fat or oil is being tested. Fat constants are divided into two classes, the **physical constants** and the **chemical constants.**

Physical Constants. Among the physical constants of fats and oils are the **specific gravity,** the **refractive index,** the **viscosity,** and the **melting point.** Since fats are mixtures of chemical compounds, the melting point is not sharp. Often the **solidification point** instead of the melting point is determined. A still better constant, known as the **titer test,** is determined from the solidification point of the mixture of free fatty acids obtained from a fat.

Chemical Constants. *Saponification Number.* The saponification number is defined as the number of milligrams of KOH necessary to neutralize the fatty acids in 1 gram of fat. It is determined by treating a weighed sample of fat with an excess of alcoholic KOH and boiling until saponification is complete. The excess KOH is then titrated with a standard solution of acid. If the amount of alkali originally added to the fat and the amount left after saponification are known, it is easy to calculate the amount neutralized by the fatty acids in the fat. It should be noted that the saponification number includes both free fatty acids and those combined with glycerol. Since each molecule of fat, regardless of its size, requires three molecules of KOH to saponify it, it is evident that the saponification number really indicates the number of fat molecules per gram of fat. The larger the fat molecule, the fewer there will be per gram of fat. The saponification number, therefore, becomes a measure of the size of the fat molecule, or more specifically the size or molecular weight of the fatty acids in the fat. A high saponification number then means a low molecular weight, and a low saponification number means a high molecular weight. Butterfat, which has a large number of low fatty acids in the molecule, has a saponification number of about 227; lard has a saponification number of about 197.

Acid Number. Sometimes it is of value to know how much free fatty acid there is in a fat. For this purpose the acid number is determined. A weighed sample of fat is dissolved in hot, neutral alcohol and titrated with standard alkali solution. If the amount of alkali taken is known, the number of milligrams of KOH required to neutralize the free fatty acid in 1 gram of fat can easily be calculated. This value is the acid number. A high acid number would be expected in a rancid fat.

TABLE 6

CONSTANTS OF FATS AND OILS*

Common Name	Specific Gravity at $\frac{15°C.}{15°C.}$	Refractive Index at 25°C.	Solidification Point, °C.	Saponification Number	Acid Number	Acetyl Number	Iodine Number	Reichert-Meissl Number	Unsaponifiable Matter
1. Beef tallow	0.895	....	31 to 38	196–200	0.25	2.7–8.6	35.4–42.3	....	....
2. Butterfat	0.907–0.912$\frac{40°}{15°}$	1.4555–1.4578$^{40°}$	19 to 24.5	210–230	0.45–35.4	1.9–8.6	26–38	17.0–34.5	0.3–0.45
3. Castor oil	0.960–0.967	1.4771	−12	175–183	0.12–0.8	146–150.5	84	1.4	0.6
4. Coconut oil	0.926	1.4547–1.4495$^{40°}$	14 to 22	253.4–262	2.5–10.0	2.3–6.9	6.2–10	6.6–7.5	0.2
5. Cod-liver oil	0.922–0.931	1.4758–1.4783	−3	171–189	5.6	1.15	137–166	0.2	0.54–2.68
6. Corn oil	0.921–0.928	1.4733	−10 to −20	187–193	1.37–2.02	7.5–11.5	111–128	4.3	1.5–2.8
7. Cottonseed oil	0.917–0.918$\frac{25°}{25°}$	1.4743–1.4752$^{15°}$	+12 to −13	194–196	0.6–0.9	21–25	103–111.3	0.95	1.1
8. Human fat	0.9179	1.459–1.4613$^{40°}$	15	193.3–199	....	....	64	0.25–0.55	....
9. Lard oil (fatty tissue)	0.913–0.915	1.4609–1.4620	27.1 to 29.9	195–203	0.5–0.8	2.6	47–66.5	....	0.6
10. Linseed oil	0.930–0.938	1.4797–1.4802	−19 to −27	188–195	1–3.5	....	175–202	0.95	0.4–1.2
11. Olive oil	0.915–0.920	1.4657–1.4667	2	185–196	0.3–1.0	10.5	79–88	0.6–1.5	0.4–1.0
12. Palm oil	0.924	1.4603–1.4639$^{40°}$	....	200–205	10	15.7	49.2–58.9	0.9–1.9	....
13. Peanut oil	0.917–0.926	1.4620–1.4653$^{40°}$	3	186–194	0.8	3.5	88–98	0.4	0.5–0.9
14. Sesame oil	0.919$\frac{25°}{25°}$	1.4704–1.4717	−4 to −6	188–193	9.8	....	103–117	1.1–1.2	0.95–1.32
15. Wool fat	0.970–0.973	1.4784–1.4822$^{40°}$	....	82–130	59.8	23	17–29	8	39–44

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* Taken from *Handbook of Chemistry and Physics* by C. D. Hodgman. Courtesy, Chemical Rubber Co.

Ester Number. If the acid number is subtracted from the saponification number, the result is the ester number. The ester number may be defined as the number of milligrams of KOH necessary to combine with the fatty acids which are in combination with glycerol in 1 gram of fat. A pure fat should have no acid number, and the ester and saponification numbers should be the same.

Acetyl Number. The acetyl number is a measure of the number of OH groups present in a fat or oil. In determining this number the fat or oil is first treated with acetic anhydride, which reacts with each OH group, introducing an acetyl group. A weighed sample of the acetylated fat is then saponified with alcoholic KOH, and the fatty acids are freed from their soaps by adding mineral acid equivalent to the KOH used in saponification. Acetic acid, being a soluble fatty acid, may then be separated from the rest by filtration and determined by titration with standard alkali. In this determination a blank must be run on the unacetylated fat, since most fats contain some soluble fatty acids other than acetic. The value obtained for the blank is subtracted from that obtained for the sample and the difference is used for calculating the acetyl number. The acetyl number is defined as the number of milligrams of KOH required to neutralize the acetic acid obtained by saponifying 1 gram of acetylated fat. Most fats and oils have a low acetyl number averaging between 3 and 15. Caster oil, which is high in ricinoleic acid, has a value of about 150.

Iodine Number. The iodine number is defined as the number of grams of iodine absorbed by 100 grams of fat or oil. Since the iodine is absorbed by the double bonds in the molecule, the iodine number is a measure of the degree of unsaturation of the fat or oil. Linseed oil has an iodine number of 170 to 200; lard has an iodine number around 50 to 70. There are several methods of determining the iodine number. One commonly used is that of **Hanus.** A small sample of oil is weighed into a bottle and dissolved in chloroform. To this is added Hanus' iodine solution, which is a solution of equivalent amounts of iodine and bromine dissolved in glacial acetic acid. After the reagent has been given 30 minutes to react, KI solution is added, and the free iodine is titrated with standard $Na_2S_2O_3$ solution, starch being used as an indicator. The KI is added to react with any free bromine in the solution forming KBr and free iodine. Thus all the free halogen in the solution is in the form of iodine when the solution is titrated with $Na_2S_2O_3$ solution. The amount of halogen in the original solution being known, it is easy to calculate the amount absorbed by the oil. It should be noted that both bromine and iodine may be absorbed by the oil. The value, however, is expressed in terms of iodine.

Unsaponifiable Matter. By unsaponifiable matter is meant that part of a fat or oil that is insoluble in water or incapable of forming a soluble soap with alkalies. Pure edible fats and oils usually contain only about 1 or 2 per cent of unsaponifiable matter. If a fat is saponified and then warm water is added to the saponified mixture, the unsaponifiable matter appears as oily drops or as a milkiness. It may be separated by extraction with petroleum ether.

The unsaponifiable fraction of fats and oils has assumed great importance in nutrition, since it has been shown that certain vitamins, which are associated with fats, are present in this fraction. The sterols, which will be discussed later, are important constituents of unsaponifiable matter.

Reichert-Meissl and Polenske Numbers. The Reichert-Meissl number, which is especially helpful in detecting adulteration of butterfat, is a measure of the amount of volatile soluble fatty acids present in a fat or oil. It is determined by saponifying 5 grams of fat with alkali, adding an excess of H_2SO_4 to liberate the fatty acids from their soaps, and distilling to remove the volatile fatty acids. The distillate is filtered, and the filtrate titrated with 0.1 *N* alkali. The Reichert-Meissl number is defined as the number of milliliters of 0.1 *N* alkali required to neutralize the **volatile soluble** fatty acids distilled from 5 grams of saponified and acidified fat.

The Polenske number is determined in the same manner as the Reichert-Meissl number, except that the insoluble volatile acids are dissolved in alcohol and titrated with 0.1 *N* alkali. The Polenske number may, therefore, be defined as the number of milliliters of 0.1 *N* alkali required to neutralize the **volatile insoluble** fatty acids distilled from 5 grams of saponified and acidified fat.

The Reichert-Meissl and Polenske numbers, as indicated above, are of value in testing for the adulteration of butter. The following table shows how three different fats compare with regard to these numbers:

	Reichert-Meissl Number	Polenske Number	Sum
Butterfat	17.0–34.5	1.5–3.0	18.5–37.5
Coconut oil	6.6–7.5	16.8–17.8	23.4–25.3
Lard	0.35	0.5	0.85

Thus it can be seen that butterfat has a high Reichert-Meissl number and a low Polenske number. Although coconut oil has about the same amount of total volatile fatty acid as butterfat, the greater proportion is in the insoluble fatty acid fraction. The quantity of volatile fatty acids in lard is small.

Physiological Importance of Fats and Oils. The fats and oils are of importance in plants and animals because they are a means of storing energy. In the animal body, fats are stored in fatty tissues which, except for the water present, are almost pure fat. Fats give about two and one-fourth times as much energy as carbohydrates or proteins. The storage of fat, therefore, is the most economical way, as far as weight is concerned, for the body to maintain a reserve energy supply.

Fat is a poor conductor of heat; hence, when stored under the skin, it serves to prevent losses of heat from the body.

As mentioned above, certain unsaturated fatty acids are indispensable in the diet; if they are lacking, a deficiency disease results.

Waxes

Like the fats and oils, the waxes are esters but differ from them chemically in that a long-chain alcohol or sterol replaces the glycerol. The following are a few of the common alcohols and acids found in waxes:

Alcohols	
Lauryl	$C_{12}H_{25}OH$
Cetyl	$C_{16}H_{33}OH$
Octodecyl	$C_{18}H_{37}OH$
Carnaubyl	$C_{24}H_{49}OH$
Ceryl	$C_{26}H_{53}OH$
Myricyl	$C_{30}H_{61}OH$
Cocceryl	$C_{30}H_{60}(OH)_2$

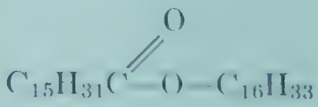
Acids	
Myristic	$C_{13}H_{27}COOH$
Palmitic	$C_{15}H_{31}COOH$
Cerotic	$C_{25}H_{51}COOH$
Melissic	$C_{29}H_{59}COOH$

In most waxes the alcohol is a long-chain monohydric alcohol, but in some it is a dihydric alcohol or sterol. Cholesterol esters, which are waxes, are found in the blood. Waxes are also found in certain insect secretions.

Waxes are much more difficult to saponify than fats. Although long boiling with alcoholic KOH will bring about saponification, they are more easily saponified by treating a solution of the wax in petroleum ether with absolute alcohol and metallic sodium. The sodium reacts with the alcohol to form sodium ethylate, which is the saponifying agent.

When waxes are saponified the fatty acids form water-soluble soaps. The long-chain alcohols formed, being insoluble in water, appear in the "unsaponifiable matter" fraction. Waxes contain from 31 to 55 per cent of unsaponifiable matter, whereas fats and oils contain from 1 to 2 per cent.

Common Waxes. **Spermaceti**, a typical wax found in the head of the sperm whale, is the cetyl ester of palmitic acid and has the following formula:



$$C_{15}H_{31}C(=O)-O-C_{16}H_{33}$$

Cetyl palmitate

Spermaceti is used in making candles.

Sperm oil is a liquid wax which occurs with spermaceti in the sperm whale. It is a valuable lubricant used for delicate instruments, such as watches. It does not become gummy, as many oils do. **Beeswax** is composed mainly of myricyl palmitate. **Carnauba wax** is found on the leaves of the carnauba palm of Brazil and is an ingredient in the manufacture of various wax polishes. Because waxes are very inert chemically, they make an excellent protective coating.

Lanolin or wool wax is obtained from wool and is used in making ointments and salves. It readily forms an emulsion with water, and for this reason makes it possible for drugs which are soluble in water to be incorporated into salves. **Chinese wax** is the secretion of an insect. Esters of cholesterol are important waxes in the blood.

Physiological Importance. Concerning the physiological significance of waxes it may be said that their most important function is as a protective agent on the surfaces of animals and plants. They are found on the surface of feathers and hair, which they help to keep soft and pliable. They prevent aquatic animals from becoming wet. On coming out of the water, a duck or muskrat will shake himself once and is apparently dry. A dog under similar circumstances will remain wet for some time.

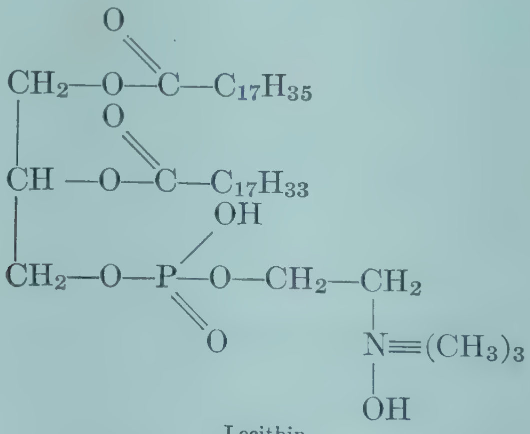
The waxy coating on the surface of plants protects them from excessive loss of moisture. Desert plants, such as the palm and cactus, can live for long periods without rain. It also protects the plant from becoming infected with fungi and bacteria which cause disease. Many fruits, such as apples and citrus fruits, have a waxy covering which prevents them from drying out and makes it possible to store them for long periods of time. The waxy covering also protects such fruits from organisms which cause rot. In picking and handling fruits to be kept for any length of time, great care should be taken to protect the waxy surface. If this surface is broken, an avenue of entrance is provided for rot-producing organisms. Care in the picking and handling of citrus fruits has proved to be a very important economic factor in the citrus fruit industry.

The Phospholipids

The **phospholipids** or **phosphatides** are a group of compounds found in every living cell. They may be defined as lipids that yield on hydrolysis an alcohol, fatty acid, phosphoric acid, and a nitrogen base. Three types of phospholipids, differing from each other in the nitrogen base present, are recognized. They are (1) **lecithins,** which contain choline, (2) **cephalins,** which contain hydroxyethylamine or serine, and (3) **sphingomyelins,** which contain choline and sphingosinol.

In our study of carbohydrates it was pointed out that, before being used by the body, the monosaccharides are first combined with phosphoric acid. Since the phospholipids are found in the body in greatest abundance in those tissues which are most active, it has been suggested that, before fats can be utilized by the body, they must first be converted into phospholipids. The presence of phosphoric acid in the molecule may convert a relatively inactive fat molecule into one that is more reactive chemically and therefore more easily metabolized by the body.

Lecithins. The best-known examples of phospholipids are the lecithins, which are found in egg yolk and the brain. Very possibly the lecithin of egg yolk has the following formula:



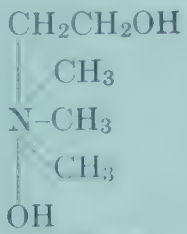
Lecithin

Thus egg lecithin is the stearic, oleic, phosphoric acid ester of glycerol in which **choline,** the nitrogen-containing compound, is in ester formation with one of the acid hydrogens of the phosphoric acid. Other lecithins may differ from this one by having different fatty acids in the molecule or by the point of substitution of the phosphoric acid in the molecule. If the phosphoric acid is tied to the end carbon of glycerol, the compound is called an **α-lecithin;** if it is tied to the central carbon atom, it is called a **β-lecithin.** Another very interesting type of lecithin is called **lysolecithin.** This is a lecithin in which the unsaturated fatty acid has been removed by hydrolysis. Lysolecithin, when introduced into the blood, causes a disintegration of the red blood cells known as **hemolysis.** Certain snake venoms contain an enzyme capable of converting lecithin into lysolecithin, and at least part of the toxicity of a snake bite may be due to hemolysis caused by lysolecithin. Snake venom also has a toxic effect on the nervous system.

Lecithins are insoluble in water but have a strong affinity for it, readily forming emulsions. It is possible that this affinity for water makes lecithin such an important constituent of protoplasm. One function attributed to lecithin is that it aids in the organization of the cell structure.

Commercially lecithin is prepared from soybeans and has several important applications. Added to the chocolate used in making candy, it prevents the formation of white spots on the surface of chocolate creams. As a constituent of oleomargarine it gives this product a consistency similar to that of butter. Added to ethyl gasoline, it inhibits corrosion of storage tanks due to lead.

Choline. Choline may be looked upon as a derivative of NH_4OH in which one hydrogen is replaced by the hydroxyethyl group and three others by methyl groups. Its chemical name then becomes hydroxyethyltrimethyl ammonium hydroxide.

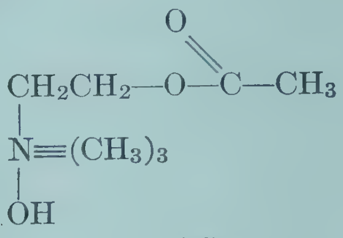


$$\begin{array}{l} CH_2CH_2OH \\ |\quad CH_3 \\ N\text{–}CH_3 \\ |\quad CH_3 \\ OH \end{array}$$

Choline

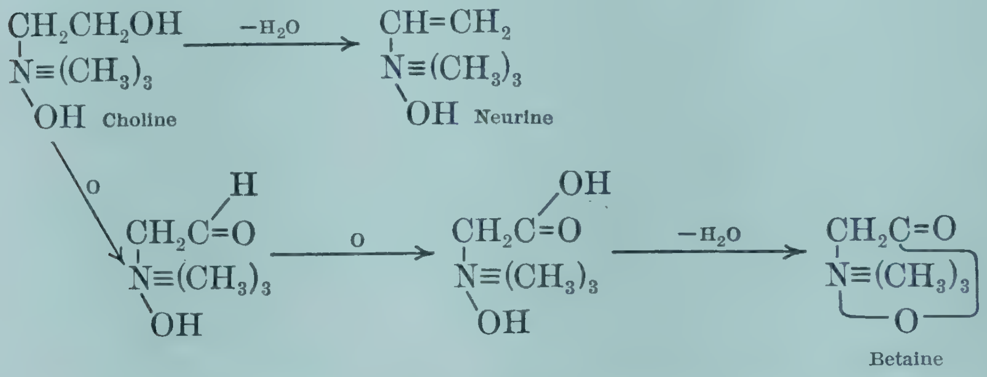
Choline has been known for a long time, but it is only within the past few years that its physiological significance has been appreciated. It now appears to be essential in order that certain physiological processes may proceed normally. In fact, if choline is eliminated from the diet, definite pathological conditions may arise. Some persons consider choline to be a vitamin, but others object to this classification, because the body appears to be able to synthesize choline, provided certain precursors are available. Among the symptoms resulting from a deficiency of choline in the diet are the development of **fatty livers** and **hemorrhage of the kidney** in rats and **perosis,** or slipped tendon, in chicks. In perosis the hock joint of the chick fails to develop normally, the tendon slips out of place, and the chick is unable to stand on his feet.

A derivative of choline known as **acetylcholine** is important because it is believed to be involved in the stimulation of muscles by nerves. The details of how acetylcholine acts are given on p. 302.



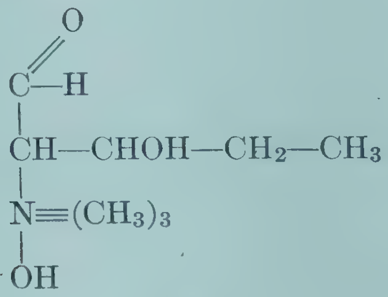
Acetylcholine

Choline is related chemically to some other interesting compounds; the relationships may be indicated thus:



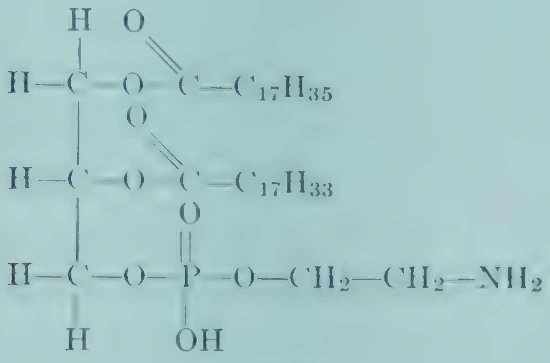
Neurine is a toxic base, being about twenty times more toxic than choline. **Betaine** is found in molasses from sugar beets and is not toxic.

Muscarine, a compound related to choline, is responsible for the toxicity of certain mushrooms. It gets its name from the fact that it is found in *Amanita muscaria,* one of the most deadly of posionous mushrooms. It has the following formula:



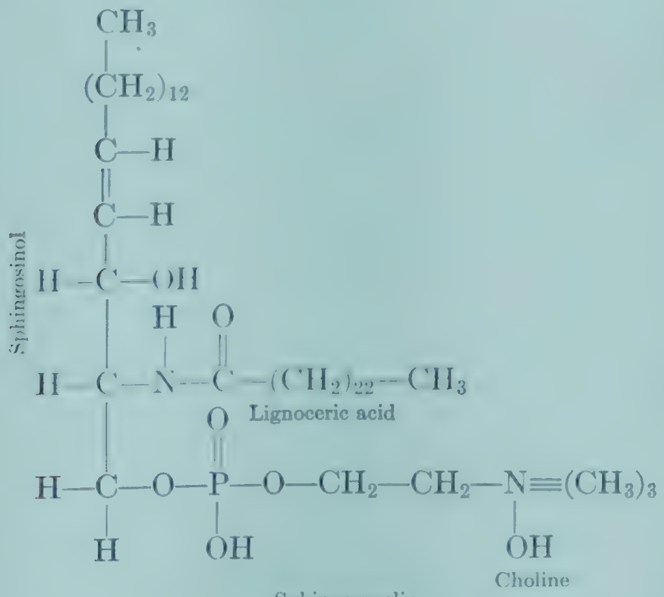
Muscarine

Cephalins. Cephalins are found in all tissues of the body but are especially prominent in the brain, from which fact the name is derived. In chemical structure they resemble the lecithins closely, the difference being that **hydroxyethylamine** ($HOCH_2CH_2NH_2$) replaces choline. In some cephalins the amino acid serine ($HOCH_2CH{\cdot}NH_2{\cdot}COOH$) replaces hydroxyethylamine. As in the lecithins, two fatty acids are present in the molecule, usually one saturated and the other unsaturated. Also there are α- and β-cephalins. The following is the formula for an α-cephalin:



α-Cephalin

Sphingomyelin. This phospholipid was first isolated from the brain by Thudichum, an early investigator of brain chemistry. It differs chemically from lecithins and cephalins in two important respects: (1) in place of glycerol its nucleus is **sphingosinol,** a dihydric amino alcohol with one double bond; (2) it contains one molecule of fatty acid, usually **lignoceric**, tied to the amino group rather than being in ester formation with an alcohol group of sphingosinol. **Phosphoric acid** and **choline** are present, linked to sphingosinol through an alcohol group. The structure of sphingomyelin is as follows:

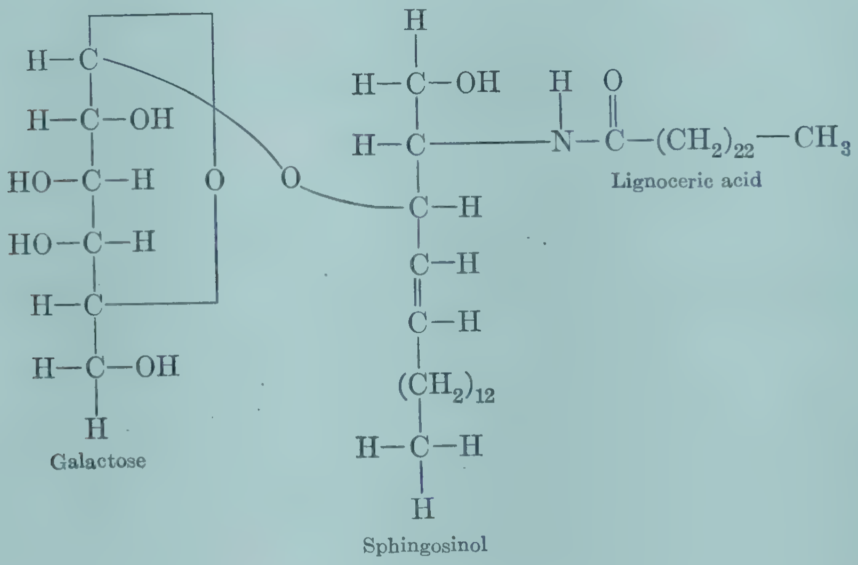


Sphingomyelin

As was true of the lecithins and cephalins, several sphingomyelins may exist, differing in the fatty acid present or the hydroxyl group of sphingosinol which is in ester formation with the phosphoric acid.

The Glycolipids

The **glycolipids,** often called the **cerebrosides,** are compounds found especially in the brain and nervous tissue, which yield on hydrolysis a fatty acid, sphingosinol, and a sugar, usually galactose. In structure they resemble the glycosides, being glycosidic derivatives of galactose. The most familiar representatives of the glycolipids are **phrenosin** and **kerasin.** Phrenosin yields on hydrolysis **galactose, sphingosinol,** and a long-chain hydroxy acid called **phrenosinic acid.** Kerasin has **lignoceric acid** in the molecule as the fatty acid. The presence of galactose in the glycolipids suggests the importance of milk sugar in the diet of infants and children during the development of the brain and nervous system.

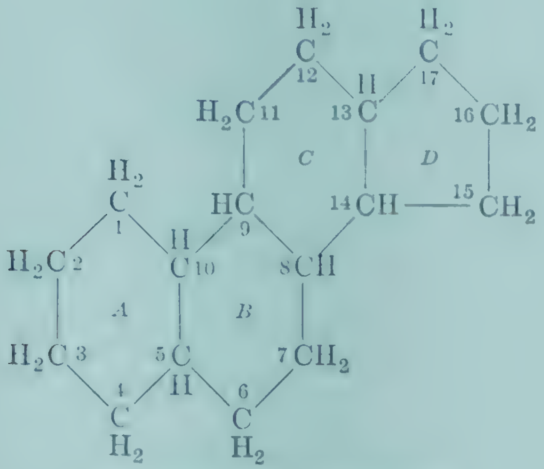


Kerasin

The Sterols

Of the derived lipids, the only group that has not been discussed is the sterols.

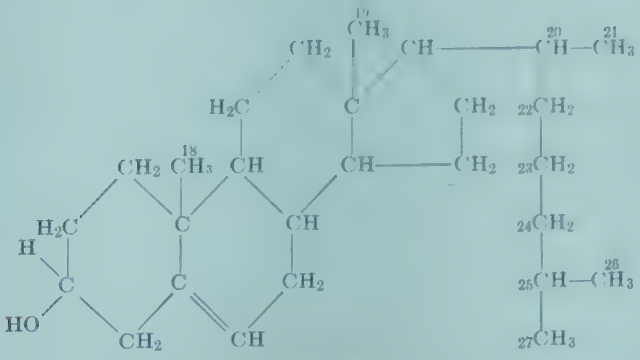
The word sterol means solid alcohol. Although the straight-chain alcohols of high molecular weight found in the common waxes are sometimes included among the sterols, it is more usual to include only cyclic alcohols which are derivatives of a cyclic hydrocarbon called **cyclopentanoperhydrophenanthrene.** The numbers in the following formula indicate positions in the molecule and facilitate the naming of derivatives. Ring *D* is the cyclopentano part of the molecule, and rings *A, B,* and *C* comprise the perhydrophenanthrene part of the molecule.


H₂
C
12
H₂
C
17
H
H₂C 11
13 C
16 CH₂
C
D
H₂
C
1
HC
9
14 CH
15
CH₂
H
H₂C 2
C 10
8 CH
A
B
H₂C 3
5 C
7 CH₂
4
H
6
C
H₂
C
H₂

Cyclopentanoperhydrophenanthrene

Five types of sterols are commonly recognized, namely: (1) the sterols proper, (2) the bile acids, (3) the genins, (4) the sex and adrenal hormones, and (5) vitamins D. It should be pointed out that not all members of all of these groups are alcohols. For this reason it has been suggested that the term **sterids** or **steroids** be used in place of sterols to designate this class of compounds.

Sterols Proper. The most common sterol is **cholesterol,** which is found in the bile and is a common constituent of gallstones. Cholesterol is also present in the brain and nervous tissue and in fact is said to be an essential constituent of all animal cells. The chemical formula for cholesterol, as given by Windaus, is:

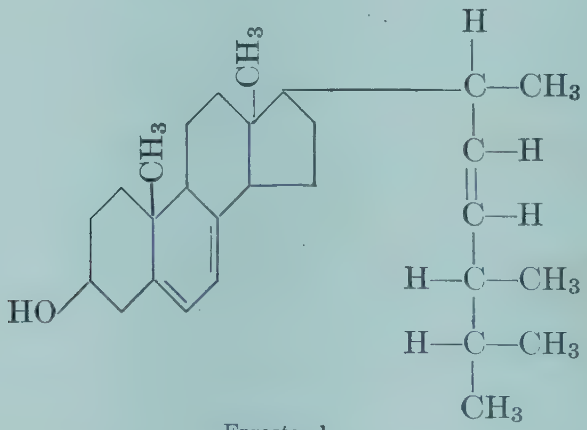

19
CH₃
CH₂
CH
20
21
CH—CH₃
H₂C
C
CH₂
22CH₂
18
CH₂ CH₃ CH
CH
CH₂
23CH₂
H₂C
C
CH
H
24CH₂
C
C
CH₂
26
25CH—CH₃
HO
CH₂
CH
27CH₃

Cholesterol

Thus it can be seen that cholesterol is a derivative of cyclopentanoperhydrophenanthrene with one double bond, one hydroxyl group, and three side chains attached to it. The various sterols differ from one another in the nature of the side chains and in the number and positions of the double bonds and hydroxyl groups.

Two reactions of cholesterol which are important and which are used in its determination are, first, that it forms an insoluble compound with the glycoside digitonin called **digitonincholesterol**; and second, that, when in chloroform solution and treated with acetic anhydride and H_2SO_4, it gives a blue-green color. **Coprosterol** is found in the feces and is a reduced cholesterol. It differs from cholesterol in that it has no double bond.

The sterols found in plants are called **phytosterols. Ergosterol,** a common phytosterol, derives its name from the fact that it was first found in the fungus ergot. It is of special interest because it has been shown to be closely related to vitamin D. It has the following simplified formula:

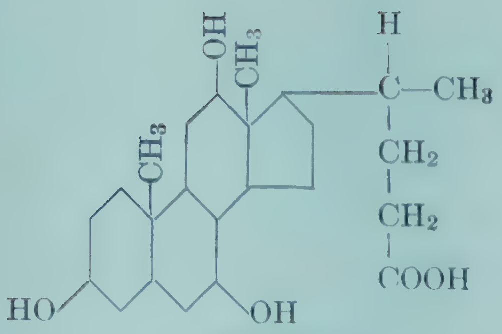


Ergosterol

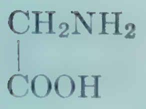
Ergosterol differs from cholesterol in that it has two more double bonds, one in the ring and one in the side chain. It also has an extra methyl group in the side chain.

Bile Acids. The bile acids are found in the bile in the form of salts known as the **bile salts.** They are very important constituents of the bile because, as will be shown later, they aid in the digestion and absorption of fats. There are two important bile acids, **glycocholic acid** and **taurocholic acid,** which are combinations of the sterol **cholic acid** with **glycine** and **taurine,** respectively. The union is between the carboxyl group of cholic acid and the amino group of glycine or taurine (see p. 131).

It should be pointed out in passing that there are several cholic acids that differ from each other in the number and position of the

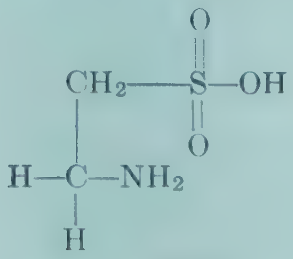


Cholic acid



$$\begin{array}{l} CH_2NH_2 \\ | \\ COOH \end{array}$$

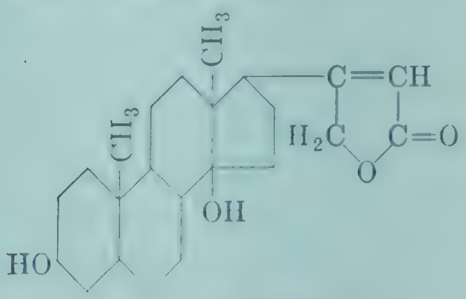
Glycine



Taurine

hydroxyl groups in the molecule. Two other important ones are desoxycholic acid, which has two hydroxyl groups, and lithocholic acid, which has only one.

Genins. The genins are important sterols found in certain glycosides, where they form the aglucone portion of the molecule. Perhaps the best examples of this group of glycosides are found in the digitalis glycosides, familiar in medicine as heart stimulants. Digitoxigenin, one of the genins found in digitalis, has the following formula:



Digitoxigenin

The chemical structure of the sex and adrenal hormones and vitamins D, the other two groups of sterols, will be discussed in Chapters 20 and 21.

Essential Oils

The essential oils are not lipids in the chemical sense but are odoriferous substances which may be distilled from flowers and other parts of plants and have many of the physical properties of oils. They are a group of heterogeneous compounds, found especially in plants, which may be extracted with ether and which form a temporary grease spot on paper. They are volatile and burn. They are used in the manufacture of perfumes and flavoring extracts and as drugs.

The term essential oil is misleading as it suggests that these compounds are very important to the plants in which they occur. Actually the name is derived from the fact that they are the substances found in the essences or volatile compounds which give many plants their characteristic odor or taste. It has been suggested that essential oils may help plants to repel harmful insects or attract useful ones. The odor of flowers may attract insects important for pollination.

The essential oils differ widely in composition. Oil of banana and oil of wintergreen are esters, the former being isoamyl acetate and the latter methyl salicylate. Oil of bitter almonds and oil of cloves are aldehydes, the former being benzaldehyde and the latter cinnamic aldehyde. Eugenol, vanillin, and thymol, found in oil of cloves, in vanilla extract, and in oil of thyme, respectively, are phenols. Oil of garlic and oil of mustard contain sulfur, the former being allyl sulfide and the latter allyl isothiocyanate. Oil of geranium and oil of rose contain geraniol, an unsaturated alcohol. Oil of pine contains pinene which is a terpene, a cyclic hydrocarbon. Camphor and menthol are, respectively, ketone and alcohol derivatives of terpene. It is thus evident that the essential oils are a heterogeneous group of compounds.

One of the most important uses of essential oils is in the production of perfume. In this industry the essential oils are extracted from flowers by exposing petals in a closed chamber to thin layers of fat on glass plates. As the essential oil volatilizes from the petals, it is absorbed by the fat. Finally, the fat is extracted with cold alcohol, which dissolves the essential oil and a small amount of the fat. When the alcohol is cooled to a low temperature the fat is frozen out. On filtering the fat is removed, leaving an alcoholic solution of the essential oil.

It is interesting to note that a similar process occurs when fats such as butter are kept in a refrigerator with certain vegetables such as onions and garlic. The essential oils of the vegetables volatilize and are absorbed by the butter giving it a disagreeable odor and taste.

REVIEW QUESTIONS

1. What are lipids?
2. Classify lipids and characterize each class.
3. Name four classes of fatty acids. Give examples of each class.
4. Give a proof for the position of the double bond in oleic acid.

5. What is meant by geometric isomerism? What other types of isomerism may exist in fatty acids?
6. What is the difference between hard and soft soaps?
7. What is Gardinol? What are its advantages compared to soap?
8. Write the formula for glycerol. Name and write the formulas for some of its oxidation products.
9. What is acrolein? How is it made from glycerol?
10. Write the formula for a fat. How do fats differ from oils?
11. What is the difference between a simple and a mixed glyceride?
12. Discuss animal fats.
13. What is oleomargarine? How does it compare with butter in food value?
14. Name three classes of vegetable oils. Give several examples of each class.
15. Discuss the hydrogenation of oils. How and why is it done?
16. Discuss the chemistry involved in the drying of oils.
17. What is boiled linseed oil? Should it be used internally?
18. Distinguish between the old and the new process for making linseed oil.
19. Name several ways of hydrolyzing a fat.
20. Write an equation showing what happens when a fat is saponified with NaOH.
21. What is lipase?
22. Distinguish between hydrolytic and oxidative rancidity. What is the chemistry involved in each type?
23. What is meant by the induction period of a fat?
24. What is an antioxidant? Name several.
25. What is the Kreis test?
26. How may fatty acids be separated into four groups?
27. Name several physical constants of fats.
28. Define and state the significance of the following chemical constants of fats: (1) saponification number, (2) acid number, (3) ester number, (4) acetyl number, (5) iodine number, (6) unsaponifiable matter, (7) Reichert-Meissl number, (8) Polenske number.
29. Discuss the physiolgical importance of fats and oils.
30. Indicate how a wax differs from a fat in chemical structure.
31. Discuss the physiological importance of waxes.
32. Name three types of phospholipids and indicate the chemical structure of each.
33. How do α-lecithin. and ß-lecithin, and lysolecithin differ from each other?
34. Name several uses of lecithin.
35. Why does snake venom hemolyze blood?
36. Write the formulas for choline and acetylcholine. What are their functions in the body?
37. What is muscarine?
38. Indicate the chemical structure of a glycolipid.
39. Name five types of compounds of biological importance that are sterols.
40. What is the hydrocarbon from which sterols are derived?
41. Indicate the chemical structure of cholesterol and ergosterol.
42. Name and indicate the chemical structure of the bile acids.
43. Why is the term "essential" applied to the essential oils?
44. Name several types of chemical compounds found in the essential oils.
45. Why is it incorrect to classify essential oils as lipids?

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5

Proteins

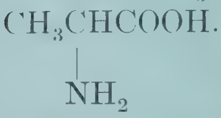
Protoplasm is usually considered as being composed primarily of protein. For this reason it is a temptation to say that proteins are the most important compounds we have to consider in our study of the chemistry of life. However, when we remember that certain carbohydrates and lipids enter intimately into the chemical structure of protoplasm and are undoubtedly essential compounds of it, it is perhaps incorrect to state that one vital constituent is more important than another.

For animals, however, proteins are more important to life than carbohydrates and lipids, because animals are unable to form new protoplasm unless protein is present in their food. Plants are able to synthesize all the compounds which make up their tissues by the process of photosynthesis, but animals must depend upon plants for their building materials. It is at least theoretically possible for an animal to live on protein alone (plus mineral salts, vitamins, and certain unsaturated fatty acids), but it is impossible for an animal to live on carbohydrate or lipid or both without protein. Thus proteins seem to have a unique function in the economy of animal life.

The most characteristic thing about the composition of proteins is that all contain nitrogen in combination with carbon, hydrogen, and oxygen. Many proteins contain sulfur and phosphorus. Some contain iron, copper, manganese, or iodine.

Proteins are extremely complicated compounds. They are made up of many simple molecules called **amino acids.** If a protein is boiled with an acid, it will hydrolyze to form amino acids. In this respect proteins resemble the polysaccharides of the carbohydrates. It will be recalled that the polysaccharides are hydrolyzed by acid to form monosaccharides or simple sugars. The simple sugars are to carbohydrate chemistry what the amino acids are to protein chemistry. In studying carbohydrate chemistry we learned that only four hexose monosaccharides commonly occur in foods. Many of the polysaccharides give only one monosaccharide on hydrolysis. This fact greatly simplifies the study of carbohydrate chemistry. When we consider the amino acids found in proteins, we find about twenty amino acids commonly present.

and a single protein may yield some of each of these amino acids on hydrolysis. It has been estimated that protein molecules such as those found in egg albumin contain in the neighborhood of 280 amino acid molecules. It is thus evident that proteins are very complex in their structure.

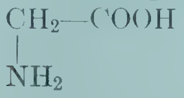
Amino Acids. Let us first consider the structure of an amino acid. An amino acid is an ordinary organic acid which has an NH_2 group in the molecule. For example, propionic acid has the formula CH_3CH_2COOH. The amino acid derived from it is  $CH_3\underset{\underset{NH_2}{|}}{C}HCOOH$. It is called α-aminopropionic acid or **alanine.** In naming acid derivatives it will be recalled that the carbon next to the carboxyl group is called the alpha (α) carbon atom. The second carbon atom from the carboxyl group is beta (β), the third gamma (γ), the fourth delta (δ), the fifth epsilon (ε), etc.

Since proteins are essential in nutrition, it is extremely important that a student become familiar with the amino acids of which proteins are composed. The amino acids have been called the alphabet of protein chemistry. Since one is continually hearing or reading about them, it is time well spent to become thoroughly familiar with their names and formulas.

The following are the common amino acids found in protein:

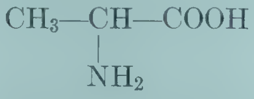
I. Aliphatic, monoamino, monocarboxylic acids.

1. Glycine or glycocoll — aminoacetic acid.



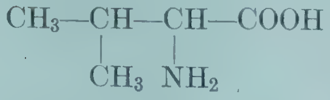
$$\underset{\underset{NH_2}{|}}{CH_2}-COOH$$

2. Alanine — α-aminopropionic acid.



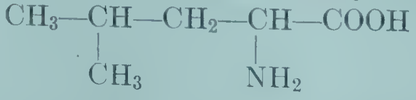
$$CH_3-\underset{\underset{NH_2}{|}}{CH}-COOH$$

3. Valine — α-animo-β-methylbutyric acid.



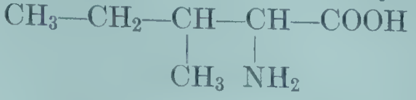
$$CH_3-\underset{\underset{CH_3}{|}}{CH}-\underset{\underset{NH_2}{|}}{CH}-COOH$$

4. Leucine — α-amino-γ-methylvaleric acid.



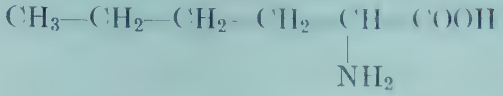
$$CH_3-\underset{\underset{CH_3}{|}}{CH}-CH_2-\underset{\underset{NH_2}{|}}{CH}-COOH$$

5. Isoleucine — α-amino-β-methylvaleric acid.



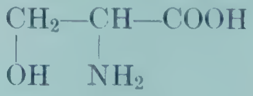
$$CH_3-CH_2-\underset{\underset{CH_3}{|}}{CH}-\underset{\underset{NH_2}{|}}{CH}-COOH$$

6. Norleucine — α-aminocaproic acid.



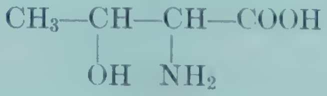
$CH_3-CH_2-CH_2-CH_2-CH(NH_2)-COOH$

7. Serine — α-amino-β-hydroxypropionic acid.



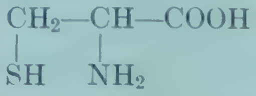
$CH_2(OH)-CH(NH_2)-COOH$

8. Threonine — α-amino-β-hydroxybutyric acid.



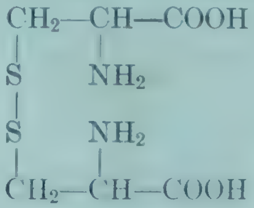
$CH_3-CH(OH)-CH(NH_2)-COOH$

9. Cysteine — α-amino-β-thiolpropionic acid.



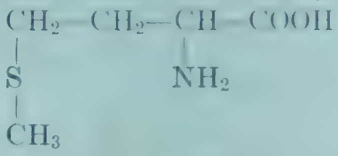
$CH_2(SH)-CH(NH_2)-COOH$

10. Cystine — di (α-amino-β-thiolpropionic acid).



$HOOC-CH(NH_2)-CH_2-S-S-CH_2-CH(NH_2)-COOH$

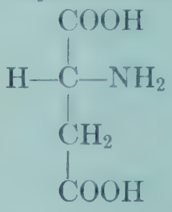
11. Methionine — α-amino-γ-methylthiolbutyric acid.



$CH_3-S-CH_2-CH_2-CH(NH_2)-COOH$

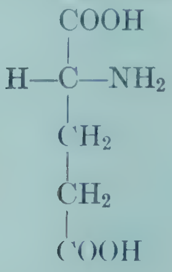
II. Monoamino, dicarboxylic acids.

1. Aspartic acid — aminosuccinic acid.



$$\begin{array}{c} COOH \\ | \\ H-C-NH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

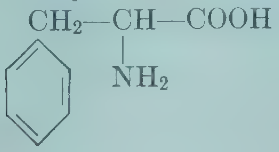
2. Glutamic acid — α-aminoglutaric acid.



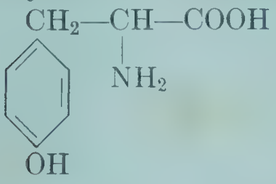
$$\begin{array}{c} COOH \\ | \\ H-C-NH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

III. Aromatic amino acids.

1. Phenylalanine — α-amino-β-phenylpropionic acid.

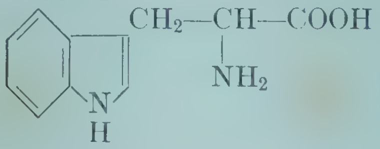


2. Tyrosine — α-amino-β-parahydroxyphenylpropionic acid.

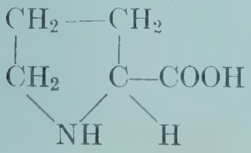


IV. Heterocyclic amino acids.

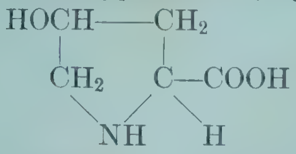
1. Tryptophan — α-amino-β-indolepropionic acid.



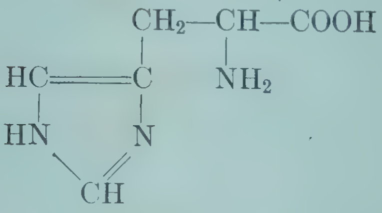
2. Proline — α-pyrrolidinecarboxylic acid.



3. Hydroxyproline — γ-hydroxy-α-pyrrolidinecarboxylic acid.

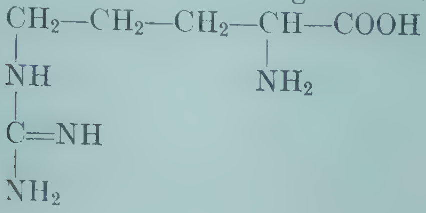


4. Histidine — α-amino-β-imidazolepropionic acid.



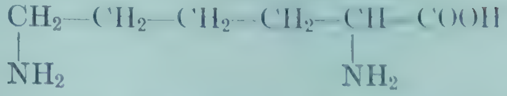
V. Diamino, monocarboxylic acids.

1. Arginine — α-amino-δ-guanidinevaleric acid.



$$\begin{array}{lllll}
\mathrm{CH_2}— & \mathrm{CH_2}— & \mathrm{CH_2}— & \mathrm{CH}— & \mathrm{COOH} \\
| & & & | & \\
\mathrm{NH} & & & \mathrm{NH_2} & \\
| & & & & \\
\mathrm{C{=}NH} & & & & \\
| & & & & \\
\mathrm{NH_2} & & & &
\end{array}$$

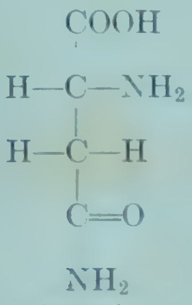
2. Lysine — α,ε-diaminocaproic acid.



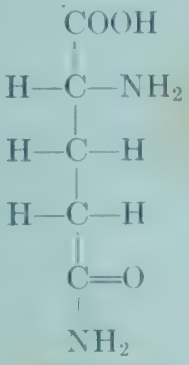
$$\underset{NH_2}{\underset{|}{CH_2}}—CH_2—CH_2—CH_2—\underset{NH_2}{\underset{|}{CH}}—COOH$$

This list of twenty-one amino acids includes all the important amino acids found in the common proteins. It should be mentioned, however, that this is not a complete list of all the known amino acids which have been isolated from proteins. A complete list would bring the total number to over thirty.

Among the amino acids not included in the above list but which should be familiar to the biochemist are **asparagine**, **glutamine**, **citrulline**, **ornithine**, and **thyroxine**. Asparagine and glutamine are the monoamides of aspartic and glutamic acids, respectively.



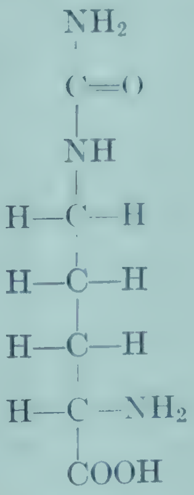
Asparagine



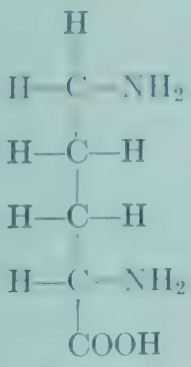
Glutamine

Asparagine is found in asparagus and glutamine in gliadin, a protein found in wheat. It is believed that they occur in the protein molecule and that they are converted to asparatic and glutamic acids, respectively, when the proteins are hydrolyzed with acid.

Both citrulline and ornithine are derivatives of arginine. These



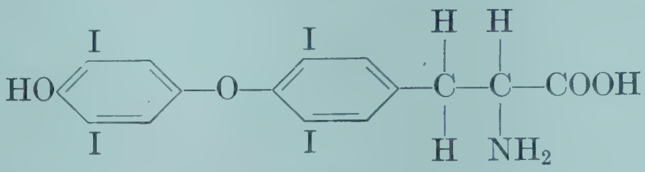
Citrulline



Ornithine

acids are of interest because they are thought to be intermediate compounds in the synthesis of urea in the liver. Citrulline has been found in watermelon juice.

Thyroxine may be considered a derivative of tyrosine containing iodine. It is the hormone produced by the thyroid gland.



Thyroxine

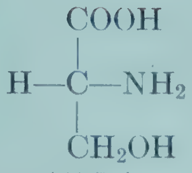
It will be noted from the formulas that all the amino acids listed are α-amino acids. Where there is more than one amino group, one is in the α position. If our list of known amino acids were complete, it would be found that all the known naturally occurring amino acids are alpha except one. The exception is β-alanine ($H_2NCH_2CH_2COOH$). Whether β-alanine occurs as such in protein or is derived from aspartic acid by the removal of carbon dioxide from the carboxyl group next to the amino group is uncertain.

Strictly speaking, not all the amino acids listed are amino acids. Proline and hydroxyproline are really imino acids, since they contain NH groups instead of NH_2 groups. However, they are usually classified as amino acids.

It will be noted from the formulas that all the amino acids listed except glycine contain an asymmetric carbon atom and therefore should be optically active and should exist in the D and L forms. In most amino acids the only asymmetric carbon atom is the α-carbon atom to which the amino group is attached. In several of the amino acids, namely, isoleucine, threonine, and hydroxyproline, there are two asymmetric carbons, and therefore more than two optical isomers are possible.

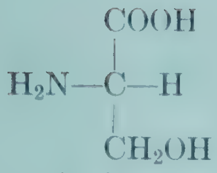
As was true of the carbohydrates, the designation D or L before the name of an amino acid does not refer to its actual rotation but rather to its relationship to D- or L-glyceric aldehyde. Since D- and L-serine are related structurally to D- and L-glyceric aldehyde, actually D- and L-serine are used as reference compounds for the amino acids. In order to indicate the rotation of an amino acid the sign (+) or (−) is placed before its name, for example, L(−)-phenylalanine. In writing formulas D may be designated by placing the NH_2 group to the right and L by placing it to the left of the α-carbon atom (see p. 141).

Most of the naturally occurring amino acids belong to the L family and are levorotatory; however, several are dextrorotatory.



$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{H{-}C{-}NH_2} \\ | \\ \mathrm{CH_2OH} \end{array}$$

D(+)-Serine



$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{H_2N{-}C{-}H} \\ | \\ \mathrm{CH_2OH} \end{array}$$

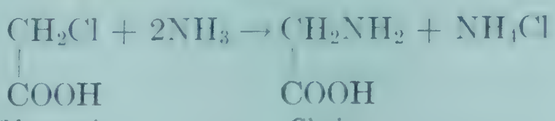
L(−)-Serine

All the amino acids listed have a common and a systematic name. The common names are the ones generally used; hence, they should be learned. If the corresponding systematic name is learned, the formula may be easily reproduced, provided simple organic nomenclature is understood.

Preparation of Amino Acids. At the present time much interest is being shown in the importance of various amino acids in nutrition. In order that such studies may be made it is obvious that a supply of the amino acids to be investigated must be available. There are two general methods used to obtain amino acids. One is hydrolysis of protein and the other is organic synthesis.

The simplest way to obtain an amino acid is to hydrolyze a protein rich in the desired amino acid and then by suitable means precipitate it. For example, cystine may be obtained by hydrolyzing hair with 20 per cent HCl. When the hydrolyzed mixture is neutralized to the proper pH (4.7), the cystine crystallizes out on standing and may be separated by filtration.

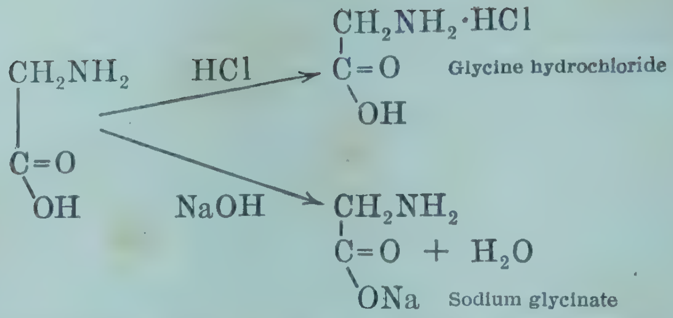
Many chemical methods have been used to synthesize amino acids. Some are simple, and others quite complex. Only one method will be mentioned here, namely, that of treating the monochloro derivative of an acid with ammonia. Glycine may be prepared by treating chloroacetic acid with ammonia, thus:



$$\underset{\mathrm{COOH}}{\underset{|}{\mathrm{CH_2Cl}}} + 2\mathrm{NH_3} \rightarrow \underset{\mathrm{COOH}}{\underset{|}{\mathrm{CH_2NH_2}}} + \mathrm{NH_4Cl}$$

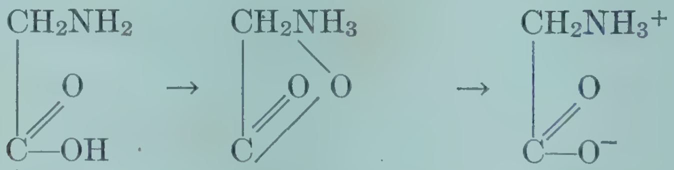
Chloroacetic acid Glycine

Chemical Properties of Amino Acids. AMPHOTERIC PROPERTIES. The carboxyl group is characteristic of organic acids and the amino group of organic bases. Since amino acids have both groups in the molecule, they would be expected to form salts with both acids and bases. Compounds which have both acidic and basic properties are said to be **amphoteric**. Monoaminomonocarboxylic acids in solution are neutral to litmus. Monoaminodicarboxylic acids in solution are acid, and diaminomonocarboxylic acids in solution are alkaline, to litmus. If **glycine** is treated with acid and with base, the following reactions take place:



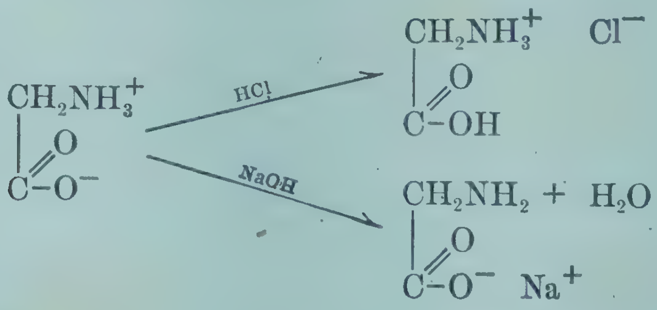
The above reactions exemplify the amphoteric properties of an amino acid.

ZWITTERIONS OR DIPOLE IONS. In the preceding section it was stated that a solution of an amino acid such as glycine is neutral. This fact might be explained on the assumption that the molecule has not ionized either as an acid or as a base. Or it might be assumed that the compound has ionized equally as an acid and as a base. Still another explanation is that the basic amino group has neutralized the acid carboxyl group to form an inner salt which is neutral. If this salt should ionize, as salts do in solution, a molecule positive at one end and negative at the other would result, thus:



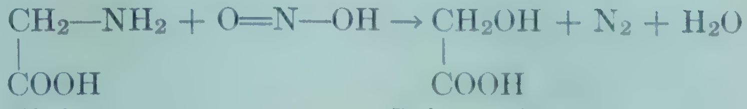
Glycine Inner salt Zwitterion

Such an ion is called a **zwitterion** or **dipole ion.** According to the zwitterion hypothesis, whole protein molecules form zwitterions as well as amino acids. Zwitterions exist at the isoelectric point of the compound from which they are formed. The reaction of a zwitterion with an acid and a base may be represented thus:



The addition of an acid to a zwitterion depresses the ionization of the carboxyl group leaving the positively charged ion to form a salt with the acid. The addition of a base depresses the ionization of the amino group leaving the negatively charged ion to form a salt with the base.

REACTION WITH NITROUS ACID. A very important reaction of amino acids is that with nitrous acid (HNO_2). Any compound containing a primary amino group will react with HNO_2 to form an alcohol, free nitrogen, and water. Glycine reacts thus:

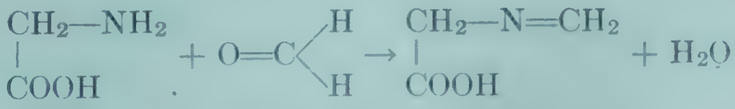


$$\begin{array}{l}CH_2-NH_2\\ |\\ COOH\end{array} + O{=}N-OH \rightarrow \begin{array}{l}CH_2OH\\ |\\ COOH\end{array} + N_2 + H_2O$$

Glycine Hydroxyacetic acid

Van Slyke has developed an apparatus for measuring the volume of nitrogen evolved in this reaction. Since one-half the nitrogen comes from amino groups, the Van Slyke apparatus is used for determining the amount of amino nitrogen in a solution. This method has been of great value in studying protein chemistry.

SÖRENSEN TITRATION. If an amino acid solution is treated with a neutral formaldehyde solution, the amino group reacts, forming a methylene compound which is no longer basic. The carboxyl group may then be titrated with standard alkali. The amount of alkali required indicates the amount of carboxyl present. This method of determining carboxyl groups in an amino acid solution is known as the Sörensen titration. The following equation represents the reaction of an amino acid with formaldehyde:



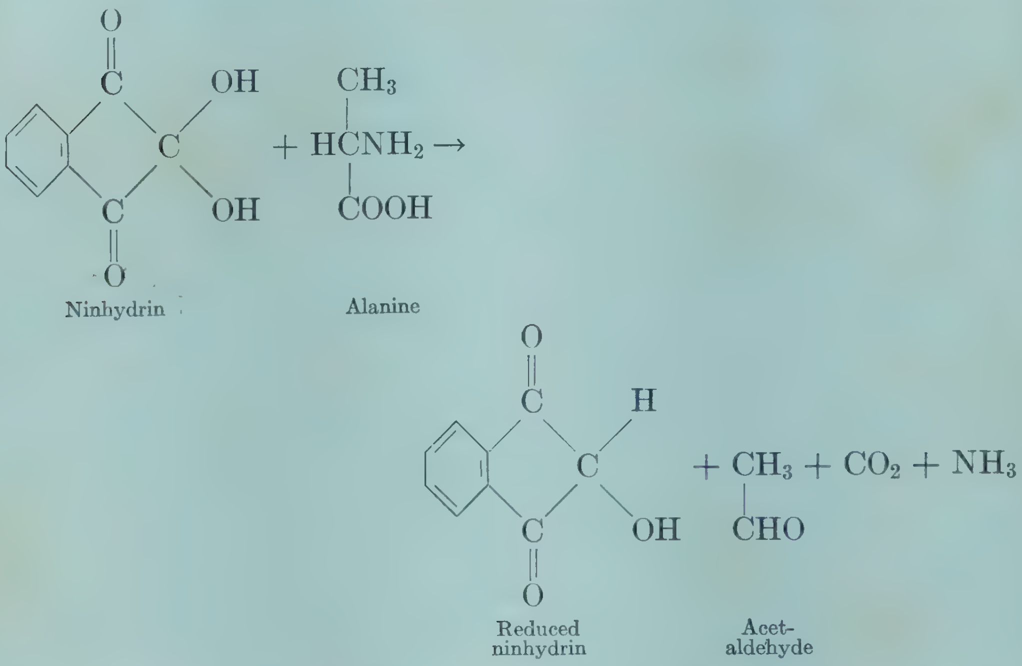
$$\begin{array}{l}CH_2-NH_2\\ |\\ COOH\end{array} + O{=}C\begin{array}{l}\diagup H\\ \diagdown H\end{array} \rightarrow \begin{array}{l}CH_2-N{=}CH_2\\ |\\ COOH\end{array} + H_2O$$

Glycine Formaldehyde Methylene glycine

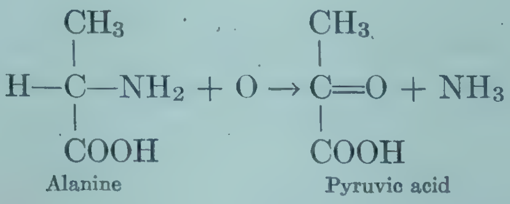
Use has been made of the Sörensen titration to determine the amount of amino acid nitrogen present in such biological materials as urine. In this method the urine is neutralized to phenolphthalein, then neutral formaldehyde is added, and the mixture is again titrated to a pink color with standard alkali. The final titration gives the data necessary for calculating the amino acid nitrogen present, since for each amino group covered up with formaldehyde a carboxyl group is set free. It should be noted that in the Sörensen titration ammonium salts react like amino groups. Hence, in determining amino acid nitrogen in the urine by this method, ammonium salts must first be removed or separately determined, and a correction applied.

REACTION WITH NINHYDRIN. When free α-amino acids are reacted with ninhydrin, one molecule of carbon dioxide is evolved for each free carboxyl group present. Proline is decarboxylated, and aspartic and glutamic acids lose both their carboxyl groups.

For the reaction with ninhydrin to occur there must be a free carboxyl group present and a free amino group tied to the carbon atom next to the carboxyl group. Since these conditions do not exist in proteins but do in free amino acids, ninhydrin is useful in determining free amino acids in the presence of proteins. Such a method has been developed by Van Slyke. In his method the solution containing free amino acids is treated with ninhydrin, and the carbon dioxide evolved is determined. The amount of carbon dioxide evolved is a measure of the amount of carboxyl present in amino acids. A modification of the method involves the determination of ammonia in place of carbon dioxide.



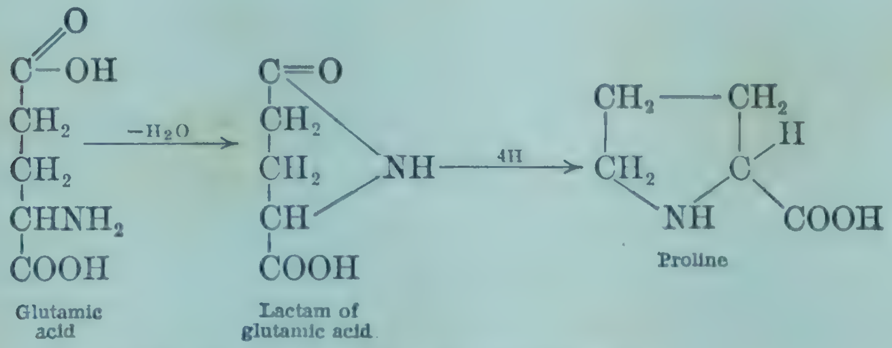
DEAMINIZATION BY OXIDATION. Of great importance to the physiologist is the reaction that amino acids undergo on oxidation. The first step in oxidation is the removal of the amino group to form ammonia and a ketone acid.



$$\underset{\text{Alanine}}{\mathrm{H{-}\underset{COOH}{\overset{CH_3}{C}}{-}NH_2}} + \mathrm{O} \rightarrow \underset{\text{Pyruvic acid}}{\mathrm{\underset{COOH}{\overset{CH_3}{C}}{=}O}} + \mathrm{NH_3}$$

This equation probably represents what happens to an amino acid when it is oxidized in the animal body.

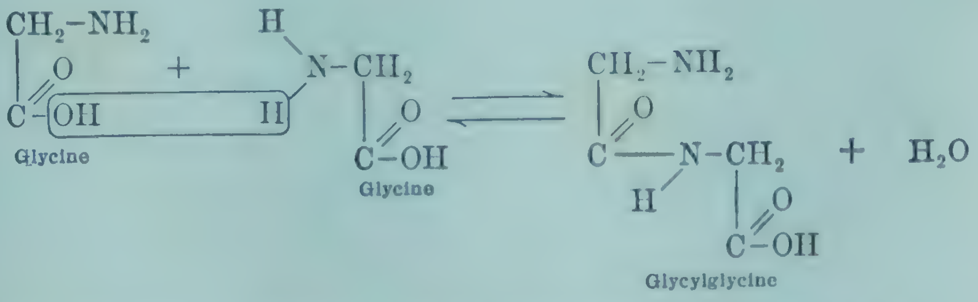
LACTAM FORMATION. A γ-amino acid will lose a molecule of water to form a heterocyclic compound called a **lactam.** This reaction may be likened to oxide-ring formation in carbohydrate chemistry. This type of reaction easily accounts for the formation of certain heterocyclic amino acids, such as **proline** and **hydroxyproline,** from **glutamic** acid. The origin of proline from glutamic acid may be represented thus:



On oxidation proline would give hydroxyproline.

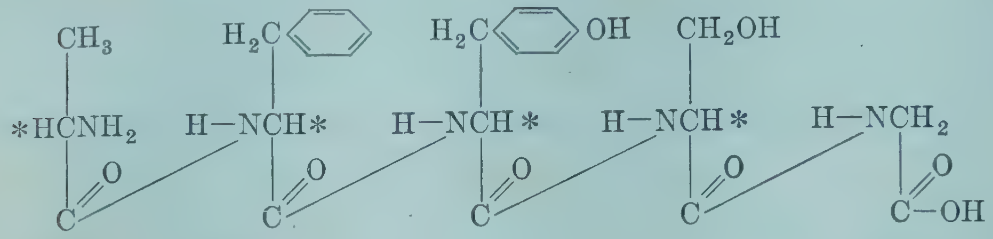
Structure of the Protein Molecule. The proteins, as has been stated, are complex structures made up of many amino acids. It should be of interest to know how the amino acids are linked together in the protein molecule. Many theories, some of which are very complicated, have been advanced to explain protein structure. That of Emil Fischer is simple and accounts for many of the known properties of proteins. Fischer believed that amino acids in the protein molecule are united by what he called the **peptide linkage.**

In the peptide linkage, water is split out from the H of the NH_2 group of one amino acid and the OH of the COOH group of another, thus:



In this manner two molecules of glycine lose a molecule of water, forming a dipeptide, which Fischer named **glycylglycine.** This dipeptide is easily hydrolyzed by the addition of water to form the amino acids from which it originated.

A more complex polypeptide would have the structure indicated by the following formula and name:



Alanyl — phenylalanyl — tyrosyl — seryl — glycine

In this formula it will be seen that a polypeptide has amphoteric properties like an amino acid. There is a free NH_2 group at the left and a free COOH group at the right end of the molecule. The formula also indicates that this polypeptide has the ability to rotate the plane of polarized light. The starred carbon atoms are asymmetric. In his laboratory Fischer succeeded in building a polypeptide containing eighteen molecules of amino acid. Later Abderhalden built a polypeptide with nineteen amino acid molecules in it.

The question naturally arises at this point as to how many amino acids there are in a single molecule of protein. The number, of course, would vary with different proteins. If the molecular weight of a protein molecule and also the average molecular weight of the amino acids found in that protein were known, the number of amino acids present could be found by dividing the former figure by the latter. There is considerable difference of opinion concerning the molecular weights of proteins, but recent work indicates that certain typical proteins have a molecular weight of about 38,000. If 38,000 is divided by the average molecular weight of the amino acids, the result is about 288. From this fact we can form a crude picture of a protein molecule. Above is the formula for a pentapeptide. If we linked fifty-eight of these pentapeptides together, we should have a rough picture of what the formula for a protein molecule may look like.

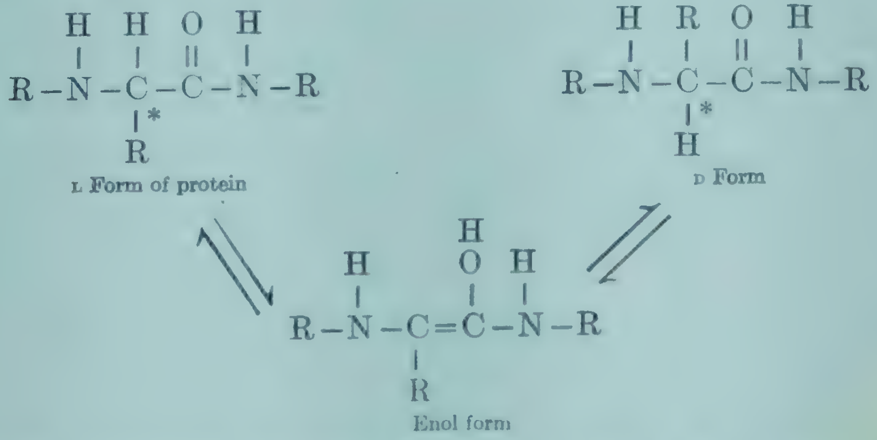
It should be pointed out that many of the amino acids contain reactive groups other than the amino and carboxyl groups. For example, cystine has an S—S group, serine has an aliphatic OH group, tyrosine a phenolic OH group, tryptophan an indole group, arginine a guanidine group, and histidine an imidazole group. It is possible that all these groups are involved in protein structure.

According to Fischer's theory, a protein molecule is composed of one long chain of amino acids. Some persons believe that the protein molecule is made up of many short polypeptide chains which are arranged side by side and held together by linkages other than the peptide linkage.

In some fibrous proteins the amino acids in the polypeptide chains are thought to be fully extended so that the fiber will not stretch without breaking; in others, such as those in hair, which will stretch, the amino acids in the chains are thought to be folded on each other as the sides of an accordion. Such fibers can be stretched without breaking the polypeptide chains. In many soluble proteins it is thought that the polypeptide chains are rolled up in globules which are oval.

X-ray studies of proteins indicate that they contain more ring structures than can be accounted for by the rings in the amino acids present. These other ring structures have been attributed to the fact that polypeptide chains are linked together in a definite pattern giving rise to rings. From what has been said it is apparent that the true nature of protein structure is a complicated affair.

Racemization of Proteins. As indicated in the previous section, proteins are optically active. When protein solutions are treated with alkali, their optical activity becomes less. This lowering of optical activity is due to racemization and is caused by a reversible enolization of optically active amino acids in the molecule. What happens to a single asymmetric carbon atom in the protein molecule may be represented thus:



In these compounds the carbon atoms designated by * are optically active. When the L form of protein is treated with alkali, the hydrogen atom tied to the asymmetric carbon atom migrates to the neighboring oxygen atom, forming an enol. This enol compound has lost its asymmetric carbon atom and is therefore optically inactive. However, this enol immediately changes back to the optically active form, but in changing back it forms equal quantities of the D and L forms. This equimolar mixture of D and L forms is what constitutes racemized protein.

It should be noted that when protein is treated with alkali the optical activity is not completely lost but is only reduced. If such a protein is completely hydrolyzed, one gets both optically active and racemic mixtures of amino acids. Thus, not all of the optically active amino acids in the molecule are racemized. It is believed that amino acids at the ends of polypeptide chains are not racemized; only those in the center of the polypeptide chains are. A dipeptide does not racemize when treated with alkali because both amino acids are end amino acids.

The racemization of proteins has been used to determine the position of an amino acid in a polypeptide. If an amino acid derived from hydrolized racemized protein is optically active, it must have been located at the end of the polypeptide chain; if optically inactive, at the center of the molecule. Racemized proteins are quite different from natural proteins in many of their biological properties. They are not digested by enzymes and do not show immunological reactions.

Classification of Proteins

I. **Simple proteins.** Naturally occurring proteins that on hydrolysis give chiefly α-amino acids or their derivatives.

- A. Albumins. Soluble in water and neutral salt solution. Coagulated by heat. Precipitated by saturation with $(NH_4)_2SO_4$; not precipitated by saturation with NaCl. Ovalbumin in egg white and serum albumin in blood.
- B. Globulins. Insoluble in water but soluble in dilute solutions of neutral salts. Precipitated by one-half saturation with $(NH_4)_2SO_4$ and by saturation with NaCl. Coagulated by heat. Serum globulin in blood, ovoglobulin in egg white, myosin in muscle, and edestin in hemp seed.
- C. Glutelins. Insoluble in water and dilute solutions of neutral salts, but soluble in dilute acid and alkali. Coagulated by heat. Glutenin in wheat and oryzenin in rice.
- D. Prolamines. Insoluble in water, but soluble in 60 to 80 per cent ethyl alcohol. Not coagulated by heat. Gliadin in wheat, zein in corn, hordein in barley, and secalin in rye.
- E. Albuminoids or scleroproteins. Insoluble in water, dilute solutions of neutral salts, acid, and alkali, and 60 to 80 per cent ethyl alcohol. Hydrolyzed by long boiling with concentrated acid solutions. Elastin in tendons, keratin in hair and horny tissue, and fibroin in silk.
- F. Histones. Soluble in water and dilute acid, and insoluble in ammonia. Not coagulated by heat. Somewhat basic in reaction be-

cause of a predominance of diamino acids. Histone in the blood corpuscles of birds.

G. Protamines. Soluble in water, dilute acid, and ammonia. Not coagulated by heat. Strongly basic in reaction because of the large number of diamino acids present. Sturin and salmin found in the sperm of fish.

II. Conjugated proteins. Simple proteins linked with nonprotein groups.

A. Chromoproteins. Protein linked with a colored compound. Hemoglobin in blood.

B. Glycoproteins. Protein linked with a carbohydrate. Mucin in saliva.

C. Phosphoproteins. Protein linked with phosphoric acid. Casein in milk and vitellin in egg yolk.

D. Nucleoproteins. Protein linked with nucleic acid. Nuclein found in the nuclei of cells.

E. Lecithoproteins. Protein linked with a phospholipid. Not a well-recognized group but undoubtedly found in protoplasm.

F. Lipoproteins. Protein linked with fatty acid. Existence doubtful, but probably found in protoplasm.

III. Derived proteins. Derivatives of proteins resulting from the action of heat, enzymes, or chemical reagents. Also artificially produced polypeptides.

A. Primary derived proteins. Derivatives of proteins in which the size of the protein molecule is not materially altered.

1. Proteans. Insoluble in water. The first products produced by the action of acids, enzymes, or water on proteins. Edestan derived from edestin.
2. Metaproteins. Insoluble in water, but soluble in dilute acid or alkali. Produced by the further action of acid or alkali on proteins. Acid and alkali metaprotein.
3. Coagulated proteins. Insoluble protein products produced by the action of heat or alcohol on protein. Coagulated egg white.

B. Secondary derived proteins. Derivatives of proteins in which definite hydrolysis has taken place. The molecules are smaller than those of the original protein.

1. Proteoses. Soluble in water, not coagulated by heat, and precipitated by saturating their solution with $(NH_4)_2SO_4$.
2. Peptones. Soluble in water, not coagulated by heat, and not precipitated by saturating their solution with $(NH_4)_2SO_4$.
3. Polypeptides. Combinations of two or more amino acids.

It will be seen from this classification that proteins are divided into three main groups on the basis of chemical differences. The **simple proteins** are the pure proteins found in nature. By pure we mean that they contain chiefly amino acids, which are the characteristic units that make up the protein molecule. The **conjugated proteins,** as the name implies, are simple proteins compounded with some nonprotein molecule. They also occur naturally, but they are not wholly protein in their make-up. The conjugated radical is not a protein and will not give amino acids on hydrolysis. The **derived proteins** are an artificial group in which are classed those derivatives produced by the action of such agencies as heat, enzymes, and chemical reagents on proteins.

Simple Proteins. The simple proteins are subdivided mainly on the basis of physical properties. Solubility is the chief factor considered. If we were to arrange the simple proteins in the order of their solubility, it would be reasonable to place the **protamines** and **histones** first and to end the list with the **albuminoids,** as is done in a classification used by English biochemists. It will be noted that the protamines, histones, and **albumins** are all soluble in water. The protamines and histones are very simple proteins as they contain only a few amino acids, mainly of the diamino type, such as **lysine** and **arginine.** The albumins differ from the protamines and histones in being more complex in structure and in being coagulated by heat. As we go down the list, we find that the **globulins** require dilute salt solution to put them into solution, the **glutelins** dilute acid or alkali, and the **prolamines** from 60 to 80 per cent ethyl alcohol. Finally we come to the albuminoids, which are very insoluble compounds.

Conjugated Proteins. The conjugated proteins are subdivided according to the nature of the nonprotein group in the molecule. If the nonprotein group is colored, we have a **chromoprotein;** if it is a carbohydrate, we have a **glycoprotein;** if it is phosphoric acid, we have a **phosphoprotein;** if it is nucleic acid, we have a **nucleoprotein;** if it is a lecithinlike compound, we have a **lecithoprotein;** and if it is a fatty acid, we have a **lipoprotein.** It should be noted that in all these groups the molecule conjugated with the protein molecule is not a protein and will not give amino acids on hydrolysis. Later, when we consider the chemistry of nucleic acid, we will learn that nucleic acid contains phosphoric acid. We have already learned that lecithin contains phosphoric acid. It should, therefore, be pointed out that not all proteins which contain phosphoric acid are phosphoproteins. The phosphoproteins do not include those proteins in which the phosphoric acid is a part of nucleic acid or a phospholipid. The lecithoproteins and lipoproteins are not well-defined groups. These groups are supposed to exist because of the intimate relations which must obtain between proteins, phospholipids, and fatty acids in protoplasm. The basic and acidic properties of lecithin and the acidic property of a fatty acid presuppose a union of these compounds with protein in protoplasm.

Virus Proteins. In 1935 Stanley made a highly important observation when he discovered that certain diseases are caused by proteins of the nucleoprotein type. Until this time it had been believed that all infectious diseases were caused by living organisms. If living organisms could not be isolated from the diseased plant or animal, it was thought that the organism was too small to be seen under the microscope. Such an organism was thought to be so small that it would pass through porcelain filters, and it was given the name **filterable virus.** A virus was then a submicroscopic pathogenic organism. Stanley worked with a serious plant disease called **tobacco mosaic.** From diseased plants he isolated a crystalline protein which even after repeated crystallization retained its ability to produce the disease in healthy plants. A short time after infection plants were found to contain large quantities of the virus protein. In other words, the virus protein is able to reproduce itself in the presence of the host plant. In this respect it appears to be living; that is, it has the power of reproduction.

The importance of Stanley's work is not so much that the causative agent of a serious plant disease was discovered, but rather that it introduced a revolutionary idea concerning the nature of the causative agent of certain diseases. It also challenged many of the old ideas about the nature of life itself. If virus proteins are living matter, it appears that the gap between living and lifeless material is almost bridged.

Studies of the properties of the virus proteins indicate that they are nucleoprotein. They differ from other nucleoproteins in that they have very high molecular weights. Those which have been studied appear to have molecular weights approximating 20,000,000.

Derived Proteins. The derived proteins are a group of what might be considered artificial proteins produced by the action of heat, enzymes, and chemical reagents on the other classes of proteins. We divide the derived proteins into two groups, depending upon whether the molecule has been altered materially in size. If the change is of such a nature that the molecule of the derived protein is of the same size as the original molecule, we have a **primary derived protein.** If the molecule has been split, the derived protein is **secondary.** If the globulin **edestin** is treated with very dilute HCl and the HCl is then neutralized, a protein called **edestan** will precipitate. It is no longer soluble in dilute salt solution. It is a primary derived protein of the **protean** type. The proteans are the first products formed when dilute acid acts on certain proteins. If egg white, which is largely albumin, is treated with a 50 per cent NaOH solution, and the resulting jelly is dissolved in water and neutralized with HCl, a precipitate forms of **alkali metaprotein.** This is an example of a **metaprotein.** The metaproteins are derived proteins which are slightly more altered from the original protein than proteans. When an egg is cooked, the protein in the egg becomes solid. The solid formed is a primary derived protein of the **coagulated** type.

In the formation of secondary derived proteins the original protein molecule is partially hydrolyzed. If the enzyme pepsin, found in the stomach, is allowed to act upon egg white, the protein in the egg white is partly hydrolyzed to form a mixture of **proteoses** and **peptones,** which are secondary derived proteins. Proteoses and peptones represent different stages in the hydrolysis of protein. The proteoses molecules are larger than those of peptone. If, to a solution of egg white that has been treated with pepsin an equal volume of saturated $(NH_4)_2SO_4$ solution is added, a precipitate of **primary proteoses** is obtained. Primary proteoses are precipitated by half-saturated $(NH_4)_2SO_4$ solutions. After filtering and saturating the filtrate with solid $(NH_4)_2SO_4$, **secondary proteoses** precipitate. When this mixture is filtered, the filtrate contains peptones. Thus it can be seen that peptones are soluble in saturated $(NH_4)_2SO_4$ solution, whereas proteoses are not. **Peptides** are combinations of two or more amino acids which result from nearly complete hydrolysis of proteins. Peptides may also be synthesized in the chemical laboratory from amino acids. Peptides are known as dipeptides, tripeptides, or polypeptides, depending upon the number of amino acids in the molecule.

Protein Allergy. When foreign proteins find their way into the body either by injection or by absorption through the respiratory or digestive tracts, important biological reactions, which are referred to as allergic reactions, may occur.

If a small amount of a foreign protein is injected into the blood stream of an animal, no visible effects are apparent; but if, after a week or so another dose of the same protein is given, the animal may have a severe reaction, often resulting in death. This reaction is known as **anaphylactic shock.** Apparently the original dose of the foreign protein has sensitized the animal to subsequent doses of the same protein. Anaphylactic shock may be prevented by giving frequent doses of the foreign protein so that the animal builds up a tolerance to the particular protein or, in other terms, is said to be desensitized.

In addition to inducing anaphylactic shock protein allergies may be manifested in other ways. Many people have in some way become sensitive to specific proteins and, when they come in contact with them later, may develop such symptoms as asthma, hay fever, eczema, headaches, and tissue swellings. We say that such a person is allergic to certain proteins. To avoid the discomfort of the allergic reactions he must avoid contact with the offending protein in the food he eats, the air he breathes, and even the clothes he wears. The proteins of milk, eggs, and wheat are the most common food allergens. The most common respiratory allergens are plant pollens. Other common allergens are wool, cat's fur, and feathers.

Protein allergy has been explained by assuming that when a foreign protein is injected into the body a sensitizing antibody, called an **anaphylactin,** is produced instead of a protective antibody. When later the same foreign protein is injected, the anaphylactin reacts with it forming a toxin, called an **anaphylatoxin,** which causes the symptoms of anaphylactic shock.

It has been suggested that the toxic substance produced in protein allergy is histamine or some similar compound resulting from the decarboxylation of histidine or some other amino acid. In support of this theory it has been shown that during anaphylactic shock histamine is present in the blood. Furthermore histamine, when injected into a normal animal, produces many of the symptoms of protein allergy.

Determination of Protein. In analyzing materials for their protein content, the **Kjeldahl method** is usually employed. In this method nitrogen is determined, and the result is multiplied by a chemical factor to convert nitrogen into protein values. The nitrogen content of the average protein being about 16 per cent, the factor 6.25 is commonly used, since $16 \times 6.25 = 100$. Since different proteins vary in their nitrogen content, other factors may be used, and so it is good practice in reporting protein values always to indicate the factor employed. In many tables there is a column headed Protein ($N \times 6.25$), meaning that the protein was calculated from the nitrogen value by multiplying by the factor 6.25. It should be pointed out that in the Kjeldahl method not protein, but nitrogen, is determined. Any nitrogen occurring in the reduced form would therefore be included in this determination. To indicate this fact the term **crude protein** has been applied to protein values obtained by the Kjeldahl method.

In the modification of the Kjeldahl method commonly used, the sample to be analyzed is heated with concentrated H_2SO_4, Na_2SO_4, and a small amount of $CuSO_4$. At first the mixture turns black because of the charring of the sample. On boiling for sometime, the solution clears up because of oxidation of the carbonaceous material. The H_2SO_4 acts as an oxidizing agent thus:

$$H_2SO_4 \rightarrow H_2O + SO_2 + O$$

The Na_2SO_4 is added in order to raise the boiling point of the mixture. The higher the temperature of the solution is, the more rapidly the oxidation takes place. The $CuSO_4$ is a catalyst which aids in the oxidation. The copper apparently acts as a carrier of oxygen from the H_2SO_4 to the material being oxidized.

After digestion is complete, the nitrogen of the sample is in the form of $(NH_4)_2SO_4$. When NaOH is added to this residue, the H_2SO_4 is neutralized and NH_3 liberated. This NH_3 is distilled into a known quality of standard acid, and from the amount of acid neutralized, as determined by titration of the excess with standard base, the amount of nitrogen present in the sample can be calculated. A piece of zinc in the distilling flask prevents bumping during the distillation.

A modification of the above method involves the collection of the ammonia in a solution of boric acid. Boric acid is a very weak acid but will react with ammonia to form ammonium borate, thereby shifting the *p*H of the solution to the alkaline side of neutrality. By the use of a special indicator the ammonia may be titrated directly with standard acid.

Amino Acid Content of Proteins. Although the Kjeldahl method gives a fairly accurate idea of the amount of protein present in the material being analyzed, it gives no information as to the amino acid content of the protein. An ideal method of protein analysis would give the amino acids present together with the percentage of each. Many attempts have been made to develop methods to analyze proteins for their amino acid content, but no single method has proved satisfactory. The difficulty arises from the fact that many of the amino acids have such similar properties that it is impossible to separate them.

Emil Fischer was one of the first to develop a method for the determination of the amino acids in a protein. He hydrolyzed the protein with HCl and converted the resulting amino acids into their ethyl esters. These he separated by fractional distillation under reduced pressure into groups of amino acid esters. Finally he hydrolyzed the esters in each group and separated the individual acids by special methods. Fischer accounted for about 65 per cent of the amino acids in a protein by his method.

Since Fischer's time many procedures have been presented for analyzing proteins for their amino acid content. Several colorimetric methods are available for the determination of certain amino acids. Other methods depend upon the precipitation, or solubility, of amino acid derivatives in various solvents. In a method developed by Brazier and Schryver the amino acids in a protein hydrolyzate are converted into their copper salts, which may be separated by a complicated procedure. In analyzing zein they accounted for 93.5 per cent of the nitrogen present.

A very useful method for analyzing proteins for certain amino acids is the **microbiological method.** This method depends upon the fact that certain microorganisms have definite amino acid requirements for growth. *Lactobacillus casei*, for example, requires at least sixteen different amino acids for growth. If this organism is inoculated into a culture medium which lacks an amino essential for its growth, no growth will occur. If a sample containing the missing amino acid is added to this culture, growth will occur, the amount of growth depending upon the amount of that amino acid present. The extent of the growth may be estimated by determining the amount of lactic acid produced by titration with standard base or by determining the turbidity of the medium which depends upon the number of microorganisms present.

With the aid of several methods of protein analysis, each of which is best suited for a particular amino acid, a considerable amount of data has accumulated as to the amino acid content of many common proteins. Table 7 gives the approximate percentage of the various amino acids found in a few typical proteins.

It will be noted from Table 7 that the proteins listed differ considerably in their amino acid contents. For this reason proteins differ in

TABLE 7

SHOWING THE APPROXIMATE PERCENTAGE COMPOSITION OF TYPICAL PROTEINS

	Casein	Gelatin	Beef Muscle	Zein (Corn)	Gliadin (Wheat)
Arginine	4.3	9.2	7.2	1.6	3.0
Histidine	2.1	0.9	2.9	0.8	2.0
Lysine	7.6	5.3	8.2	—	1.3
Tyrosine	6.7	0.3	4.4	5.4	3.1
Tryptophan	1.2	—	1.4	0.1	1.0
Phenylalanine	5.0	2.4	5.0	7.0	—
Cystine	0.35	0.2	1.1	0.9	2.6
Methionine	3.4	1.1	3.4	2.4	3.0
Threonine	3.8	1.7	5.0	2.5	3.0
Serine	7.7	3.7	5.5	—	—
Leucine	9.7	3.6	7.7	25.0	—
Isoleucine	6.3	1.2	6.3	4.0	—
Valine	6.5	2.7	5.8	3.0	—
Glutamic acid	23.3	11.6	15.6	36.6	47.0
Asparatic acid	6.1	9.6	6.1	3.4	1.4
Glycine	0.5	26.6	5.1	—	1.8
Alanine	5.5	10.4	5.0	10.0	5.8
Proline	8.0	17.2	—	12.0	13.0
Hydroxyproline	?	15.0	—	—	—

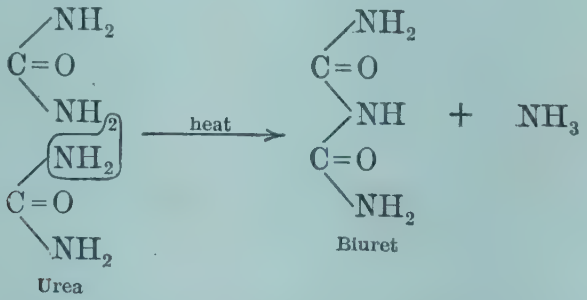
From *Introduction to Agricultural Biochemistry* by R. Adams Dutcher, Clifford O. Jensen, and Paul M. Althouse, John Wiley & Sons, New York, 1951. Courtesy of R. A. Dutcher.

their nutritive value. Glutamic acid is the predominant amino acid in all the proteins listed except gelatin. We shall learn later that glutamic acid plays an important role in protein metabolism. If the columns in Table 7 are totaled, values of over 100 will be obtained in some cases. This may be accounted for by the water added to each peptide linkage when the protein is hydrolyzed.

Color Reactions of Proteins

Before the subject of color reactions of proteins is discussed, it should be made clear that these are specific tests, not for proteins, but for certain structures commonly found in proteins. Therefore ordinarily, one positive color test is not conclusive proof of the presence of protein. To prove the presence of protein several color tests should be run, and tests should be selected which respond to a variety of characteristic groups of proteins.

Biuret Test. If to a protein solution some strong alkali and then a few drops of dilute $CuSO_4$ solution are added, a violet color is produced. This is called the biuret test, because an organic compound called **biuret** gives the same test. Biuret may be prepared by heating urea; NH_3 is given off, and the residue is biuret.



It will be noticed in the formula that the linkage of two molecules of urea in biuret is similar to the peptide linkage in proteins. Proteins then give the test because of the **peptide linkages** which they contain. It should be pointed out that dipeptides do not give the biuret test, but all other polypeptides do. Since the biuret test is a test for peptide linkage, it is evident that it is a very good one for proteins, being a test for one of the most characteristic structures in all protein molecules. One of the most valuable uses of the biuret test is in connection with the hydrolysis of proteins. It is often important to know when hydrolysis is complete. If the biuret test is negative, hydrolysis is complete, at least to the dipeptide stage.

Millon's Test. Millon's reagent is a mixture of mercuric nitrate and nitrite. It is made by dissolving mercury in HNO_3. If Millon's reagent is added to a protein solution, the protein is first precipitated as a mercury salt. On heating, the precipitate turns a flesh color if the test is positive. Only proteins containing **tyrosine** give a positive test. The hydroxyphenyl group of tyrosine is the structure responsible for this test. Thus Millon's test is for tryosine in the protein molecule.

Xanthoproteic Test. The word xantho means yellow, so this test might be called the yellow protein test. If a protein solution is treated with HNO_3, the protein is precipitated; and on heating it dissolves, giving a lemon-yellow color. When the acid is neutralized with alkali, the color changes to a burnt orange. This test is positive for proteins containing amino acids with the benzene ring, such as **tyrosine, phenylalanine,** and **tryptophan.** The test is especially good if tyrosine is present. The benzene rings are apparently nitrated, and the nitrated compounds give the burnt-orange color on treatment with alkali.

Hopkins-Cole Test. If to a protein solution in a test tube a few drops of a solution of glyoxylic acid ($CHO \cdot COOH$) are added and then a layer of concentrated H_2SO_4 is placed under this solution, a violet ring will appear at the juncture of the two liquids if **tryptophan** is present in the protein. The indole group of tryptophan is responsible for this test. Apparently the aldehyde group of the glyoxylic acid aids in the conversion of indole to the violet compound responsible for the color. The Hopkins-Cole test is, then, a test for tryptophan.

Liebermann's Test. If concentrated HCl is added to a solid protein, the mixture is boiled, and then a few drops of sucrose solution are added, a violet color appears if **tryptophan** is present in the protein. This is similar to the Hopkins-Cole test, the aldehyde coming from the action of the HCl on the sugar.

Acree-Rosenheim Test. In this test the protein solution is treated with a few drops of very dilute formaldehyde solution and then layered with concentrated H_2SO_4. A violet ring appears if **tryptophan** is present. The formaldehyde is the aldehyde here. A very important application of this reaction is in testing for formaldehyde in milk. Unscrupulous milk dealers sometimes prevent their milk from spoiling by adding small quantities of formaldehyde as a preservative. If such milk is heated with HCl, a violet color develops. The protein in the milk, together with the added formaldehyde, makes possible a positive Acree-Rosenheim test upon the addition of acid and heating.

Ehrlich's *p*-Dimethylaminobenzaldehyde Test. Ehrlich suggested the use of *p*-dimethylaminobenzaldehyde as the aldehyde in testing for tryptophan. In this test the protein solution is boiled with concentrated HCl and then a few drops of *p*-dimethylaminobenzaldehyde, dissolved in 10 per cent H_2SO_4, are added. A red to violet color develops if

tryptophan is present. The color changes to blue upon the addition of a few drops of 0.5 per cent $NaNO_2$ solution. This test is used in bacteriology in testing for indole production by bacteria.

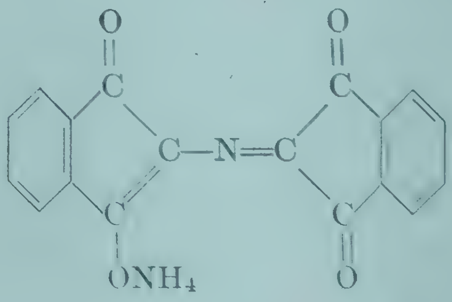
Ehrlich's Diazo Test. This test is positive if **histidine** or **tyrosine** is present. The reagent is made by mixing $NaNO_2$ solution with sulfanilic acid dissolved in HCl solution. Upon adding to a protein solution and making alkaline with NH_4OH, a red to orange color results. Histidine gives a red to orange color; tyrosine, a lighter orange color.

Reduced Sulfur Test. If a protein solution is boiled with KOH and then treated with lead acetate solution, a black precipitate is formed if sulfur-containing amino acids such as **cystine** and **methionine** are present. The strong alkali removes sulfur from these amino acids, forming K_2S, which with lead acetate forms PbS, a black compound. Methionine does not give the test unless the protein solution is first heated with a mineral acid.

Molisch's Test. If a protein solution containing sugar, either in solution or in the molecule as a glycoprotein, is treated with Molisch's reagent and then layered with H_2SO_4, a violet ring will develop. It will be recalled that the Molisch test is a test for any carbohydrate. If dealing with pure protein solutions, this is a good test for a **glycoprotein.**

Ninhydrin Test. In addition to being used as a reagent for the quantitative determination of free carboxyl groups in solutions of amino acids, ninhydrin gives an important color reaction with compounds containing **α-amino groups**. For the reaction to occur the compound must contain at least one free amino group and one free carboxyl group. This is an extremely delicate test, to which proteins, their hydrolytic products, and α-amino acids react. For certain amino acids the test is positive in dilutions as high as 1 part in 100,000 parts of water.

When ninhydrin is added to a protein solution and the mixture is heated to a boil, a lavender color appears on cooling. The color is due to the formation of a complex compound with the following formula:



Precipitation of Proteins

There are several ways in which proteins may be precipitated. Explanations of why they precipitate are based on their colloidal and chemical properties.

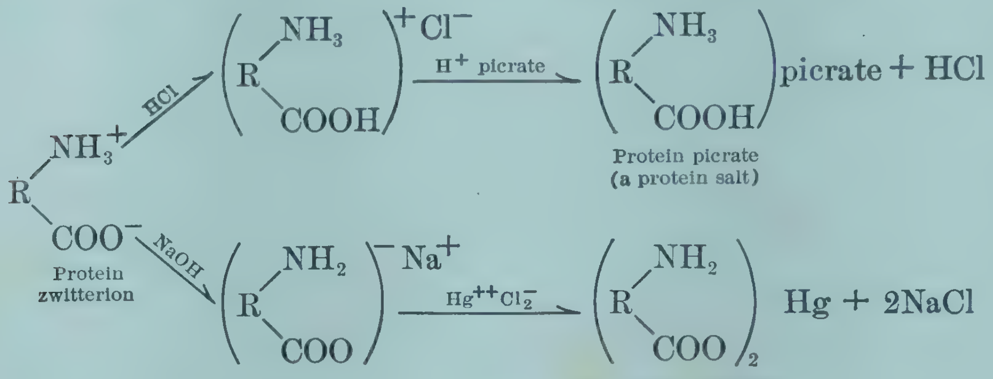
Colloidal Explanation of Protein Precipitation. Proteins are colloidal substances and remain in colloidal dispersion because of electric charges on the colloidal particles. The charge may be either positive or negative, depending upon the hydrogen-ion concentration of the solution. In neutral solution most proteins have a negative charge. As acid is added, the negative charge is reduced and finally becomes positive. The point at which positive and negative charges are equal is called the isoelectric point, and it is at this point that proteins are most easily precipitated. Sörensen has prepared egg albumin in a pure crystalline form by bringing the solution to the isoelectric point. The casein of milk is easily precipitated by adding sufficient acid to bring the solution to a *p*H of about 4.7, the isoelectric point of casein.

Proteins that have a negative charge may be precipitated by adding a positive colloid, like colloidal iron. In such a case we have mutual precipitation of the two colloids.

Many salts, such as $HgCl_2$ and $PbCl_2$, and many acids, such as picric and phosphotungstic acids, precipitate proteins. According to colloidal theory such precipitations may be explained on the hypothesis that the positive charges of the Hg^{++} and Pb^{++} neutralize the negative charges on negative colloidal protein particles or that the negative charges on the picrate and phosphotungstate ions neutralize the positive charges on positive colloidal protein particles.

Since proteins are hydrophilic colloids, the fact that they take up large quantities of water aids in their dispersibility. When large quantities of salts, such as $(NH_4)SO_2$, are added, this water is removed from the colloidal particles, and the protein is precipitated. This process is spoken of as salting out. Alcohol precipitates proteins in a similar manner.

Chemical Explanation of Protein Precipitation. In discussing the reactions of amino acids, it was pointed out that proteins, as well as amino acids, exist in solution as zwitterions at their isoelectric points. In acid solution the protein part of the molecule becomes a positive ion, and in basic solution a negative ion. Therefore in acid solution a protein will react with acid radicals to form salts which are called **protein salts.** In basic solution the negative protein radicals will react with positive radicals to form salts called **proteinates.** If the radicals which combine with the protein radicals are of the right kind, the resulting compounds are insoluble and will precipitate. The following diagram, in which R is a protein molecule, illustrates what has just been said.



Mercuric proteinate
(a proteinate)

Other acids which precipitate proteins as protein salts are **tannic, phosphomolybdic, phosphotungstic, chromic,** and **trichloroacetic acids.** In general, it may be said that protein must be on the acid side of its isoelectric point to be precipitated as a protein salt. Other metals which precipitate proteins as proteinates are **copper, iron, manganese, aluminum, lead, nickel, platinum,** and **gold.** Such alkaloids as **quinine** and **strychnine, basic dyes,** and **basic proteins,** such as the protamines, also precipitate proteins as proteinates. In general, to precipitate a protein as a proteinate, the protein must be on the basic side of its isoelectric point.

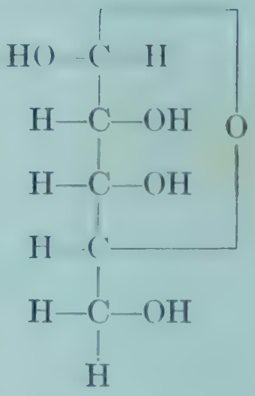
The chemistry of the precipitation of proteins is not so simple as the foregoing equations would indicate. Experiment has shown that proteins do not react with metals and acids in stoichiometric proportions, and it is a question whether they form true salts. A rational explanation of how metals and acids act in precipitating proteins must include their effect on the colloidal properties of the protein.

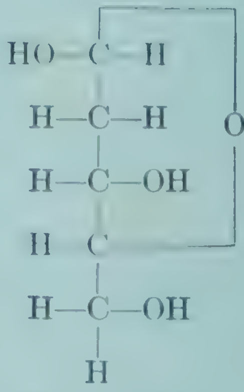
Nucleoproteins

The nucleoproteins are so called because they were first isolated from cell nuclei and were thought at that time to be found only in the nucleus. We now know that nucleoproteins are found also in the cytoplasm of cells. Much of the early work on nucleoproteins was done on material obtained from the heads of fish sperm and on white blood cells in pus, both of which are composed chiefly of nuclei.

Nucleoproteins are conjugated proteins. On partial hydrolysis they yield a protein and nuclein. Nuclein on further hydrolysis yields a protein and nucleic acid. Nucleic acid on further hydrolysis yields phosphoric acid, a sugar, **pyrimidines,** and **purines.** Before we can consider the structure of nucleic acid, it is necessary to consider the sugars present and the chemistry of the pyrimidines and the purines.

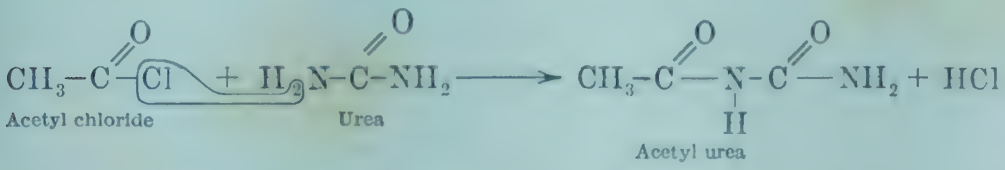
Sugars. Two pentose sugars have been isolated from nucleic acid, namely, **D-ribose** and **2-desoxy-D-ribose.** Both of these sugars have the furanose structure. D-Ribose is found in nucleic acid obtained from the cytoplasm and 2-desoxy-D-ribose in nucleic acid obtained from the nuclei of cells.


β-D-Ribose


β-2-Desoxy-D-ribose

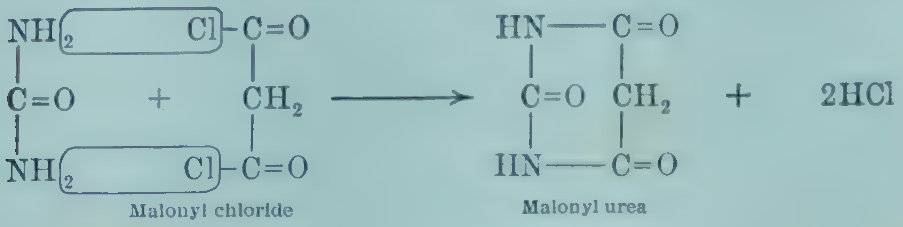
Two kinds of nucleic acid are now recognized depending upon the sugar present in the molecule. One is called **ribose nucleic acid** and the other **desoxyribose nucleic acid.** Ribose nucleic acid is also called yeast nucleic acid or plasmonucleic acid; desoxyribose nucleic acid is also called thymus nucleic acid or chromonucleic acid.

Pyrimidines. Both the pyrimidines and purines are derivatives of urea called **ureids.** Urea will react with acetyl chloride to form a compound called **acetyl urea,** thus:

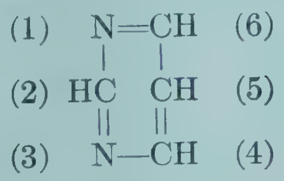


$$CH_3-\overset{O}{\overset{\|}{C}}-Cl + H_2N-\overset{O}{\overset{\|}{C}}-NH_2 \longrightarrow CH_3-\overset{O}{\overset{\|}{C}}-\underset{H}{N}-\overset{O}{\overset{\|}{C}}-NH_2 + HCl$$

With the acid chlorides of dibasic acids, both NH_2 groups of urea react, and cyclic ureids are formed. The cyclic ureid of malonic acid is **malonyl urea** and is closely related to pyrimidine.

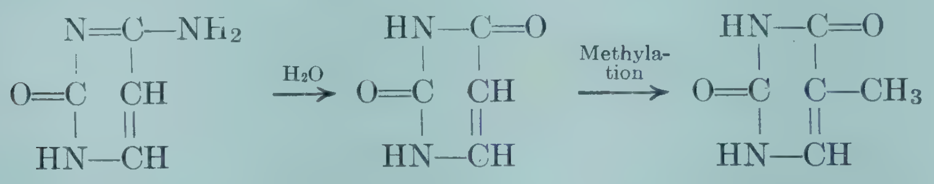


Reduced malonyl urea is pyrimidine, which has the following formula:



Pyrimidine

The numbers indicate positions in the molecule; they are used in naming pyrimidine derivatives. The pyrimidines found in nucleic acid are **cytosine, uracil,** and **thymine.**



Cytosine
(2-oxy-6-aminopyrimidine)

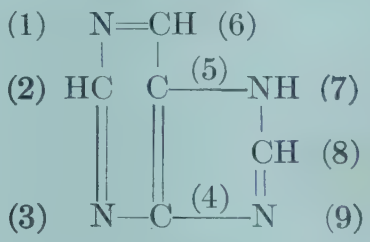
Uracil
(2,6-dioxypyrimidine)

Thymine
(2,6-dioxy-5-methylpyrimidine)

Uracil differs from cytosine in that the NH_2 group is replaced by oxygen. Thymine is uracil with a methyl group in the 5 position.

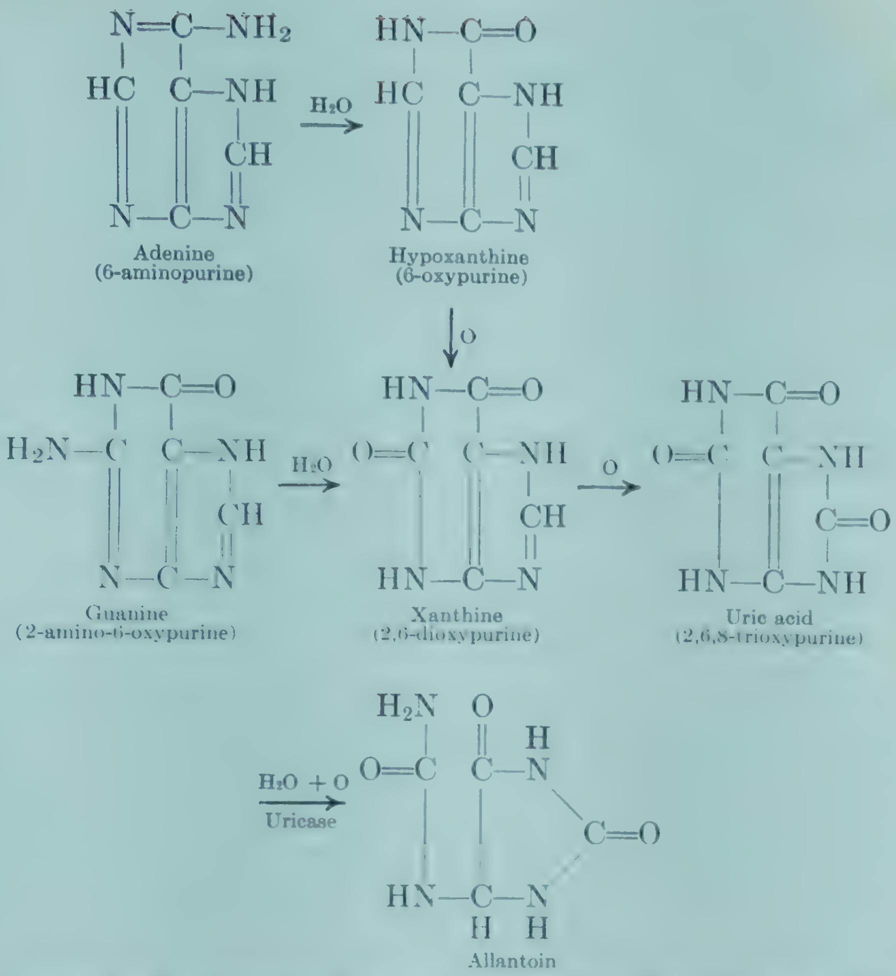
The two types of nucleic acid differ with regard to the pyrimidines present in the molecule. Ribose nucleic acid contains cytosine and uracil, whereas desoxyribose nucleic acid contains cytosine and thymine.

Purines. The parent substance of this group of compounds is **purine.** Purine may be looked upon as pyrimidine with a urea molecule forming another ring by being attached to the 4 and 5 positions, thus:

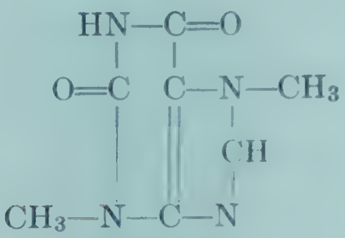


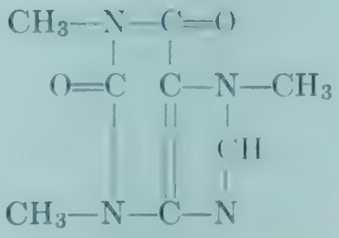
Purine

The purines found in nucleic acid are **adenine** and **guanine,** which are amino purines. On hydrolysis these purines yield **hypoxanthine** and **xanthine,** respectively. On oxidation, hypoxanthine yields xanthine, which in turn yields **uric acid,** the end product of purine metabolism in man, the anthropoid ape, and the Dalmation coach hound. In other mammals the end product of purine metabolism is allantoin. In birds and reptiles uric acid is the main nitrogenous constituent of urine. In these animals uric acid is synthesized from other substances besides the purines in the food.


H₂O
Adenine
(6-aminopurine)
Hypoxanthine
(6-oxypurine)
O
H₂O
O
Guanine
(2-amino-6-oxypurine)
Xanthine
(2,6-dioxypurine)
Uric acid
(2,6,8-trioxypurine)
H₂O + O
Uricase
Allantoin

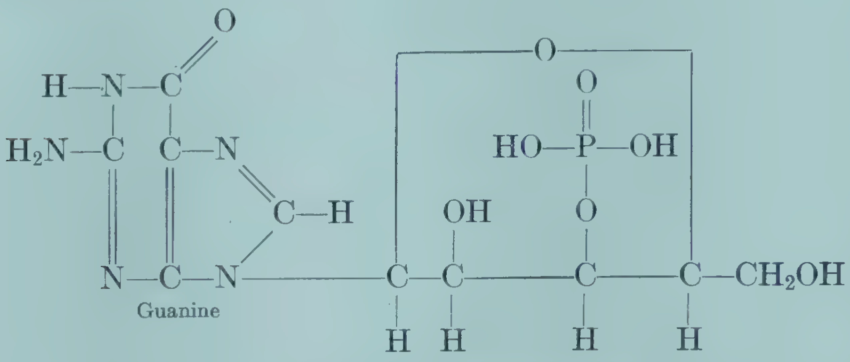
While we are considering the purines, mention should be made of two **methylpurines**, which, though not found in nucleic acid, are important. They are **theobromine**, found in cocoa, and **caffeine**, found in tea and coffee.


Theobromine
(2,6-dioxy-
3,7-dimethylpurine)


Caffeine
(2,6-dioxy-
1,3,7-trimethylpurine)

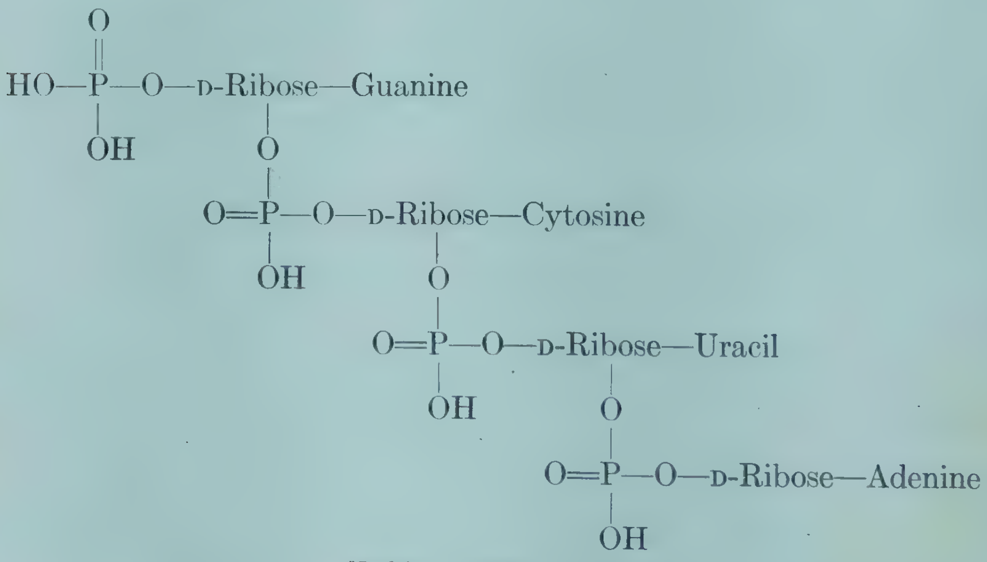
It will be noted that both theobromine and caffeine are methyl derivatives of xanthine. Caffeine has a pronounced action on the heart and is a brain stimulant. Because of its action on the brain, a person may be unable to sleep after drinking coffee at night. Caffeine is often used in medicine as a **diuretic.** A diuretic stimulates the kidneys in their production of urine.

Nucleic Acid. Levene and Jacobs, working with nucleic acid from yeast, isolated a compound which contained phosphoric acid, a pentose sugar called D-ribose, and guanine. They named this compound **guanylic acid.**



Guanylic acid
(a mononucleotide)

Guanylic acid is a **mononucleotide.** If the H_3PO_4 is removed by hydrolysis, a **nucleoside** called **guanosine** is obtained. Guanosine may be hydrolyzed to form D-ribose and guanine. According to Levene and Jacobs, nucleic acid is a tetranucleotide composed of four nucleotides, each differing from the other in that it contains a different purine or pyrimidine. Levene has suggested the following structure for such a tetranucleotide.



Nucleic acid (tetranucleotide)

If nucleic acid is a tetranucleotide it should have a molecular weight of about 1300. Actual molecular-weight determinations of various nucleic acids prepared from different sources have given in most cases values much higher than 1300; values as high as 1,000,000 have been obtained. These high molecular weights have been explained by assuming that nucleic acid is a poly- rather than a tetranucleotide. Some believe that the polynucleotide is actually a polymerized tetranucleotide and that the latter is the actual fundamental unit in the nucleic acid molecule.

It will be noted from the preceding formula that a tetranucleotide contains five acid hydrogens of the phosphoric acids in the molecule. It is likely that in nucleoprotein the protein is attached to one or more of these acid hydrogens. The proteins in nucleoprotein are often protamines, which are basic in reaction. Possibly nucleoprotein is a salt of the basic protein and nucleic acid.

REVIEW QUESTIONS

1. What is an amino acid?

2. Given the common names for the amino acids, give their systematic names and their formulas.

3. Name two amino acids which, strictly speaking, are not amino acids.

4. In naming an amino acid, how may one indicate whether it is dextro- or levorotatory?

5. What amino acids are taken as reference standards for determining the configuration of other amino acids?

6. Give two methods for preparing an amino acid.

7. Write equations showing what is meant by the amphoteric properties of an amino acid.

8. What is a zwitterion or dipole ion?

9. Write an equation showing what happens when an amino acid is treated with HNO_2.

10. Discuss the chemistry involved in the Sörensen titration.

11. How does ninhydrin react with amino acids?

12. Show by equation how an amino acid may be oxidized in the body.

13. What is a lactam? How may proline be related to glutamic acid?

14. How are amino acids linked together in the protein molecule? Write the formula for a polypeptide made up of several different amino acids. Name the polypeptide.

15. What is the average molecular weight of a protein molecule? How many amino acid molecules are there in such a protein molecule?

16. Explain the chemistry involved in racemization. Are all the amino acids in a protein molecule racemized when the protein is treated with alkali?

17. What is meant by racemized protein?

18. Name the three main classes of proteins and characterize each class.

19. Classify simple proteins and indicate the solubility of each class.

20. What is a chromoprotein? Name two important phosphoproteins.

21. What is the difference between primary and secondary derived proteins?

22. How may proteoses be separated from peptones in solution?

23. Discuss virus proteins.

24. What is meant by protein allergy? How may it be explained?

25. Name the reagents used in the Kjeldahl method and state the function of each.

26. Why is the factor 6.25 used in calculating protein values?

27. What is meant by the term crude protein?

28. Discuss the methods used in determining the amino acid content of proteins.

29. What is the most abundant amino acid present in most proteins?

30. Name the color reactions for proteins. State how each test is made. What is the appearance of a positive test in each case, and what structure in the protein molecule is responsible for each test?

31. Name a color test for proteins which would be useful in indicating the complete hydrolysis of a protein.

32. How may formaldehyde be tested for in milk?

33. Give a colloidal and a chemical explanation for the precipitation of proteins.

34. Name two types of nucleic acid. How do they differ in composition?

35. Name the pyrimidines and the purines found in nucleic acid and write their formulas.

36. Name two methyl purines and write their formulas.

37. What is the end product of purine metabolism in man? In other mammals?

38. What is a diuretic?

39. Distinguish between a nucleotide and a nucleoside.

40. Indicate the chemical structure of nucleic acid.

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6

Mineral and Organic Foods

In the foregoing chapters we have considered the chemistry of some of the more important organic constituents of the body. There remains to be considered the inorganic constituents, which although present in relatively small amounts are nevertheless very important.

It is evident that the material substance out of which the body is made must be supplied to the body in the form of food. It is also evident that the body must have some outside source of energy to supply the needs for work and for maintaining body temperature, which is usually higher than that of the surrounding atmosphere. This energy comes from the oxidation of foods. Foods must also supply the vitamins and minerals which are essential for life. A food may be defined as any substance that, when absorbed by the body, may be used for building new tissue or repairing old, for furnishing energy, for the synthesis of essential compounds, or for supplying minerals and vitamins to the organism.

Water and Mineral Foods

Water. At first thought it may seem strange to speak of water as a food, but in view of the fact that the body is composed of about two-thirds water and that a food is any substance used by the body for building tissue it is obvious that water is a very important food. In fact, from the standpoint of maintaining life water is our most important food. Experiments have shown that animals may live for more than 100 days without organic foods but that they die in 5 to 10 days when deprived of water.

It has been estimated that the average person consumes from 2 to 5 liters of water per day. Much of this water is contained in the foods we eat. Fruits and vegetables contain from 80 to 90 per cent of water; milk, 87 per cent.

Besides the water which is consumed along with foods considerable quantities are produced in the body when foods are oxidized. For example, when one molecule of glucose is oxidized, six molecules of water are produced. It has been estimated that a person producing 2400 Calories per day obtains about 300 ml. of water as a result of the oxidation of food. Such water is often spoken of as **metabolic water.**

Drinking water is usually not pure water but contains important minerals in solution. Hard waters contain large quantities of calcium salts. In some regions magnesium salts are present in such large quantities that the water is decidedly laxative in its action. In extensive areas of the United States drinking water is lacking in iodine; a deficiency of iodine is considered an important factor in the development of simple goiter.

Water serves some very important functions in the body. In it the food materials are dissolved and carried to all parts of the body. The waste products of the cells are removed and excreted by means of water. Many of the reactions taking place in the body, such as those involved in digestion and metabolism, are hydrolytic in nature and thus require water. Also many reactions are ionic in character; and, since ionization takes place in water solution, the presence of water is an important aid to such reactions. Because of its high specific heat and high heat of vaporization, water plays an important role in regulating body temperature.

Bound Water. A study of the manner in which water exists in protoplasm has revealed that it may exist either in the free state or in combination with certain of the constituents of protoplasm, usually proteins, in the form of bound water.

Bound water differs from free water in that it is combined with the constituents of protoplasm by either physical or chemical means. Therefore bound water does not separate easily from protoplasm by freezing at low temperature or by evaporation at high temperature or under dry conditions.

Bound water is of special interest in connection with the ability of plants to resist low temperatures and drought. Winter wheat is planted in the fall, and produces its crop the following summer. A severe winter will kill many varieties of winter wheat. A hardy variety of winter wheat contains three or four times as much bound water as a nonhardy variety. Drought-resistant grasses may contain ten times as much bound water as non-drought-resistant varieties. The determination of bound water in plants has been of great value to plant breeders in their development of winter-hardy and drought-resistant varieties of plants.

Inorganic Elements. The inorganic elements found in the body are for the most part very common in nature. There is a striking similarity between the salts of sea water and the inorganic constituents of protoplasm. This fact has sometimes been interpreted as evidence that life originated in the sea.

The common inorganic elements in the body are **sodium, potassium, magnesium, calcium,** and **iron,** together with **chlorides, sulfates,** and **phosphates.** Others, found only in traces and often spoken of as trace elements, are **copper, cobalt, manganese, zinc, iodine,** and **fluorine.**

Although the inorganic elements are present in the body in relatively small amounts, they are nevertheless very important. In fact, most of them are known to be essential for life. Calcium, magnesium, phosphate, and carbonate are found in bones; and phosphates and bicarbonates are important buffers in the blood and aid in the regulation of its *p*H. Inorganic salts keep blood globulins in solution. Calcium is essential for blood clotting, and iron is a necessary constituent of the hemoglobin molecule. Sulfur is found in thiamine, insulin, and glutathione; phosphorus, in nucleoproteins and phospholipids. From these few examples it is clear that the mineral elements must be important to life.

Determination of Ash. An important determination in the analysis of a food is the estimation of the amount of ash it contains. In determining ash the sample is heated to a high temperature, so that all the organic matter is oxidized and volatilized and the mineral matter remains. During ashing the nature of the mineral matter of a food may be considerably altered. For example, the sulfur of proteins appears in the form of inorganic sulfates, and the salts of organic acids appear as carbonates. Mineral elements may even be lost during ashing. If there are insufficient metallic elements to combine with the acids formed during ignition, some of the acids may be lost in such forms as SO_2 and HCl. Potassium salts may be lost because of volatilization at high temperatures. However, roughly speaking, the amount of ash in a food is a measure of its mineral content.

Sodium, Potassium, and Chlorine. One of our most abundant sources of sodium and chlorine in the diet is common salt, which is **sodium chloride.** This statement does not mean that plant and animal foods do not contain these elements, but rather that they are present in them in limited amounts, especially in plant foods, where potassium salts predominate. Foods of animal origin are richer than plant foods in sodium. The ingestion of large quantities of potassium salts increases the elimination of sodium salts in the urine, and vice versa. Thus on a vegetable diet there is more need for salt than on a mixed diet. Perhaps the reason we use so much salt in our diet is to make up for the deficiency of sodium in the vegetables which we eat. A normal mixed diet contains an adequate supply of sodium and chlorine for health. The average adult consumes about 10 grams of sodium chloride per day, and his daily requirement is probably much less.

The importance of salt in the diet is indicated by the fact that herbivorous animals, such as deer, travel long distances to reach salt licks. Hunters often place blocks of salt in the neighborhood of their cabins to encourage deer to stay in the vicinity. In early Roman history we read of salt being used in place of money. The word salary comes from the Latin word for salt. The expression, "He isn't worth his salt," means that the person isn't worth his salary. Governments have placed a tax on salt with the idea of taxing a commodity that everyone uses.

Both sodium and potassium are essential for life. Although these elements are quite similar in chemical properties, one will not replace the other in the diet. In the blood and other tissues of the body, potassium is concentrated in the cells, whereas sodium predominates in the plasma and intercellular fluid. Sodium bicarbonate is an important buffer in the blood and contributes to the alkalinity of the saliva and the intestinal and pancreatic juices. In the blood it also functions in the transportation of carbon dioxide, which is largely in the form of sodium bicarbonate. Perhaps the most important use of the chloride ion is for the production of the HCl of gastric juice.

The amounts of sodium and potassium in the blood and tissues are controlled by hormones produced by the adrenal glands. In Addison's disease, an adrenal insufficiency, there is a loss of sodium and a retention of potassium by the body. The disease has been treated by feeding a diet high in sodium and low in potassium or by supplying the missing adrenal hormones.

Most of the salt in our diet is eliminated from the body in the urine. In hot weather, however, much may be eliminated in perspiration. Excessive perspiration, such as occurs in men working at high temperatures, may remove sufficient quantities of sodium chloride from the body to cause collapse. This reaction may be prevented by drinking a 0.25 per cent solution of salt in place of water.

Calcium. Most of the calcium of the body is found in the bones, where it occurs together with magnesium as phosphates and carbonates. In the blood it is found in the plasma rather than in the cells, 100 ml. of normal serum containing about 10 mg. of calcium.

Variations in the calcium values of blood serum are found in certain diseases. Low serum-calcium values are associated with nervous irritability and in some cases may result in muscle spasm and convulsions. Low serum-calcium values may occur in rickets, where there is a deficiency of vitamin D in the diet. Vitamin D is essential for both the normal absorption and the metabolism of calcium. If the parathyroid gland becomes too active, calcium is withdrawn from the bones, and blood-calcium values may be very high; if the gland is underactive, these values may be low. In dairy cattle, milk fever is caused by a lowering of the calcium content of the blood because of loss of calcium in the milk of high-producing cows.

Calcium is essential for the clotting of blood. Clotting can be prevented by adding an oxalate to freshly drawn blood to precipitate the calcium.

Calcium is widely distributed in foods. One of the best sources of calcium in the diet is milk, where it occurs as inorganic salts and in combination with casein. Other good sources are egg yolk and vegetables. Cereals and lean meat are poor sources of calcium. Many fresh vegetables, although rich in calcium, are poor sources of this element because of the presence of organic acids, such as oxalic, which react with calcium to form insoluble salts which are not absorbed. According to the 1948 report of the Committee on Food and Nutrition of the National Research Council, a normal adult should receive in his diet 1.0 gram of calcium per day. During pregnancy and lactation this amount should be increased to 1.5 to 2.0 grams. Children should have from 1.0 to 1.2 grams, and adolescents from 1.0 to 1.4 grams per day.

About 70 per cent of the calcium taken into the body is eliminated in the feces. The remaining 30 per cent is eliminated in the urine as salts of the inorganic acids.

Magnesium. Most of the magnesium in the body is found in the bones. It also occurs in the other tissues of the body. Blood serum contains from 1 to 3 mg. per 100 ml., and muscles 21 mg. per 100 grams. Perhaps the most significant occurrence of magnesium is in the chlorophyll molecule, which is essential for photosynthesis, a most important biochemical reaction. Magnesium is also associated with certain enzyme systems involved in carbohydrate metabolism and fermentation.

Magnesium is widely distributed in foods, and there is little danger of not receiving an adequate supply of it in a normal diet. This element is essential. McCollum has shown that rats fed on a diet low in magnesium become nervous, develop tetany, and die. The average adult requires about 0.25 gram of magnesium per day. Magnesium is eliminated from the body both in the urine and in the feces.

Magnesium compounds are used in medicine as laxatives. Milk of magnesia is a suspension of magnesium hydroxide in water. It is used for the neutralization of gastric acidity and as a mild laxative. Epsom salt or magnesium sulfate is a strong laxative. The laxative action of magnesium salts is said to be due to the fact that they are absorbed very slowly and therefore remain in the intestine as a concentrated solution with high osmotic pressure. This concentrated solution draws water from the intestinal lining, thus flushing out the intestinal tract.

Iron. The main occurrence of iron in the body is in the **hemoglobin** molecule, which is found in the red blood cells. Hemoglobin functions in the blood as a carrier of oxygen. Hemoglobin is a conjugated protein made up of **heme,** which contains ferrous iron, and **globin,** a protein. Heme is said to be present in all living cells as a component of enzymes concerned in biological oxidations. Thus iron is widely distributed in living matter.

One of the main effects of a lack of iron in the diet is the development of a condition known as **nutritional anemia.** Nutritional anemia often occurs in infants who are kept too long on a milk diet. Since milk contains very little iron, in infant feeding it is customary to supplement milk with iron-containing foods, such as egg yolk.

According to the 1948 report of the Committee on Food and Nutrition of the National Research Council, the average adult should receive 12 mg. of iron per day. During pregnancy and lactation this amount should be increased to 15 mg. Children should receive from 6 to 12 mg. per day, and adolescents 15 mg.

Perhaps the best source of iron in the diet is egg yolk. Other good sources are meat, green leafy vegetables, beans, and peas. Milk, white flour, and fruits are poor sources of iron. In medicine iron is often prescribed in the form of salts. Ferrous salts are used, since this is the form in which iron is most easily absorbed from the intestinal tract.

The percentage of iron in a food is not always an index of the value of the food as a source of iron. All the iron of eggs is available, whereas only about 50 per cent of the iron of meat is available. We formerly heard that spinach was an excellent source of iron, but now we know that less than 25 per cent of this iron is available. One would expect blood to be an excellent source of iron. Actually blood is a poor source, because iron in the form of heme is not easily utilized. In general it may be said that in nutrition inorganic forms of iron are more available than organic forms.

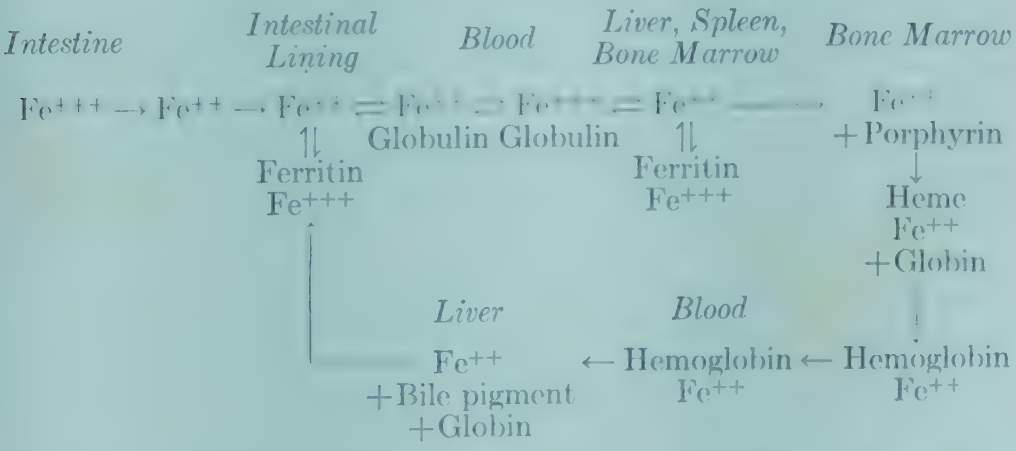
It should be pointed out that traces of copper in the diet are essential for the proper utilization of iron. This requirement will be discussed under copper.

Iron differs from most other mineral elements in that once it is absorbed it is largely retained by the body and is not excreted as other elements are. A 24-hour sample of urine contains only about 1 mg. of iron. Little is excreted into the intestine; that which is found in the feces is that which has never been absorbed from the food. During hemorrhage iron is lost as hemoglobin. To regenerate hemoglobin iron must be absorbed.

The iron in foods may be either ferrous or ferric. In the intestinal tract ferric iron is reduced to ferrous before being absorbed. In the intestinal lining some of the ferrous iron is oxidized to ferric iron which combines with a protein called apoferritin to form **ferritin.** Ferritin contains about 23 per cent of iron and is considered a storage reservoir for iron in the body. Ferrous iron that is not oxidized in the intestinal lining passes on into the blood stream where it combines with a globulin to form a ferrous iron complex which is partially oxidized to a ferric iron complex. As the blood passes through the liver, spleen, and bone marrow more ferritin is formed and stored for future use.

As hemoglobin is formed ferrous iron combines with porphyrin to form heme, which in turn combines with the protein globin to form hemoglobin. As the concentration of ferrous iron in the blood decreases, ferritin decomposes forming ferric iron and apoferritin. The ferric iron is reduced to ferrous iron, thus maintaining a uniform concentration of ferrous iron in the blood.

Hemoglobin is found in the red blood cells, which live for about 4 months. When they disintegrate their hemoglobin decomposes to form heme and globin. Heme next decomposes liberating iron, which is retained by the body and used over again, and bile pigments, which are excreted by the liver in the bile. What has just been said may be represented diagrammatically as follows:


Intestine
Intestinal Lining
Blood
Liver, Spleen, Bone Marrow
Bone Marrow
Fe+++ → Fe++ → Fe++ ⇌ Fe++ ⇌ Fe+++ ⇌ Fe++ ——→ Fe++
Globulin Globulin
Ferritin
Fe+++
Ferritin
Fe+++
+Porphyrin
Heme
Fe++
+Globin
Liver
Blood
Fe++
+Bile pigment
+Globin
← Hemoglobin ← Hemoglobin
Fe++
Fe++

Sulfur. Most of the sulfur in the body originates from the proteins of foods, where it exists in the amino acids **cystine** and **methionine.** Sulfur is found in the body in several important compounds, such as glutathione, the bile salts, insulin, thiamine, biotin, and the proteins of cartilage, hair, and feathers. When birds produce new feathers after molting the sulfur requirement in the diet is high. The same is true for laying hens since considerable sulfur is required for egg production. In human nutrition there is little likelihood of a deficiency of sulfur if the protein content of the diet is adequate.

During metabolism most of the sulfur is oxidized to sulfate and is secreted in the urine as **inorganic sulfates** or in combination with organic radicals as **etheral sulfates.** Some is secreted in an unoxidized form known as **neutral sulfur.** The formation of etheral sulfate is one method the body has of detoxifying poisonous compounds resulting from the intestinal putrefaction of proteins.

Since the sulfur in the body originates from proteins, the metabolism of sulfur will be discussed in more detail in Chapter 14 on protein metabolism. At this point it is sufficient to mention that sulfur is essential in the diet in the form of the amino acid methionine.

Phosphorus. Although most of the phosphorus in the body is found in the bones as an inorganic complex containing $Ca_3(PO_4)_2$, it is also widely distributed in the body in other compounds. It is found in nucleoproteins, phospholipids, and the hexose phosphates. The union of lipids and sugars with phosphoric acid appears to be a very important stage in their metabolism. Phosphoric acid is present in certain enzyme systems associated with carbohydrate metabolism. Creatine phosphate, found in muscle, appears to be an essential factor in muscular contraction. In the blood the alkali phosphates are important buffers that assist in regulating its *p*H.

One of the best sources of phosphorus in the diet is milk, where it is found in the phosphoprotein casein. Egg yolk, cheese, meat, and legumes are rich sources of phosphates. Wheat bran and cereals are rich in phosphorus, but much of it is in the form of phytin, an organic complex which is poorly utilized. These foods, therefore, are not considered good sources of phosphorus. In general it may be said that phosphorus is abundant in many foods and that there is little likelihood of a deficiency of this element in our diet.

According to Sherman, an adult requires 0.88 gram of phosphorus per day. Growing children require 1.3 grams per day. During pregnancy and lactation women require still more. The requirement for an adult can be met by consuming 1 quart of milk per day.

One of the most common results of a deficiency of phosphorus in the diet is rickets, a disease characterized by poor calcification of the bones. This disease, as has been pointed out, is also related to the amount of calcium and vitamin D in the diet. In rickets the amount of phosphorus in the blood serum is usually low.

Copper. Although copper is not present in the hemoglobin molecule, it has been found that traces of copper in the diet are essential for normal hemoglobin formation. An adult requires from 2 to 2.5 mg. of copper per day in his diet. In certain regions where the copper content of the soil is low nutritional anemia is common among cattle on pasture.

In some of the lower forms of life, such as the lobster, **hemocyanin,** a copper-protein complex, is the respiratory pigment found in the blood. Hemocyanin has a blue color, and thus the blood of a lobster is blue rather than red. Copper is also present in several oxidase enzymes.

Cobalt. Cobalt also appears to be essential for blood production, at least for certain species of animals. In certain areas of Florida and Australia cattle and sheep on pasture develop nutritional anemia, which may be cured by feeding small amounts of cobalt. Horses fed on the same pasture do not develop the disease.

Cobalt is a constituent of vitamin B_{12}, which is effective in the treatment of pernicious anemia, a disease characterized by a greatly reduced number of red blood cells. Perhaps cobalt functions in nutrition as a component of this vitamin. It has been shown that the feeding or injection of cobalt salts in rats and rabbits will cause an increase in the number of red blood cells. It is probable that cobalt will be found essential for the proper nutrition of all species of animals which have hemoglobin in their blood.

Manganese. Manganese is thought to play a part in normal reproduction. On a manganese-deficient diet there is reproductive failure in both male and female rats. Hens on a low-manganese diet produce eggs that hatch poorly. Manganese also appears to function in normal bone development. Chicks on a low-manganese diet develop **perosis,** a disease also associated with diets deficient in choline. (See p. 125.) Young animals deprived of manganese grow slowly. Manganese appears to play some part in certain enzyme systems involved in carbohydrate metabolism.

Although little is known about the requirement of manganese in human nutrition it has been suggested that the daily diet of children should include about 0.25 mg. per kilogram of body weight. Good sources of manganese are beet tops, lettuce, and wheat bran; fair sources are beets, spinach, and whole wheat; meat and fish are poor sources.

Zinc. Zinc is a constituent of an enzyme called **carbonic anhydrase,** which is found especially in the red blood cells and is responsible for the conversion of carbonic acid into carbon dioxide and water. Zinc also appears to be a constituent of **insulin,** a hormone essential for normal carbohydrate metabolism. Rats fed on a zinc-free diet grow slowly and produce an abnormal fur. Zinc is considered to be an essential element in nutrition.

Iodine. The adult human body contains about 25 mg. of iodine, 15 mg. of which are found in the thyroid gland where it is used in the synthesis of **thyroxine,** the active constituent of the internal secretion of that gland. Often, if there is too little iodine in the diet, **simple goiter** develops; it is said that proper attention to iodine in the diet is a sure preventive. Iodine is especially important in the diet during pregnancy. The daily requirement of iodine for normal persons is 0.05 mg. A common means of supplying it at the present time is through the use of iodized salt.

Sea water is rich in iodine, and sea foods are excellent sources of iodine in the diet. Plants grown near the sea are usually richer in iodine than plants grown inland. Simple goiter is more common inland than in regions close to the sea. For more information on the relation of iodine to simple goiter see Chapter 20.

Fluorine. Fluorine is found in the teeth and bony structures of the body. It is believed to contribute to the hardness of teeth. In regions where the fluorine content of the drinking water is low dental caries is common, whereas in regions where the fluorine content is higher dental caries is much less prevalent. Many cities are adding fluorides to their water supplies in order to aid in preventing tooth decay. The application of a solution of sodium fluoride to the surface of the teeth of children has been found to be helpful in preventing tooth decay. Since sodium fluoride is poisonous great care must be taken in its use. Too much fluorine in the drinking water will cause white, chalky areas to appear on the teeth which later become stained brown producing a condition known as "mottled enamel."

Other Trace Elements. Besides the trace elements mentioned above many other elements have been found in traces in the body. Little is known about their value. Among those that have been identified are **selenium, boron, lead, tin, silicon, molybdenum, arsenic,** and **titanium.**

In certain areas of South Dakota and Wyoming a cattle disease known as "alkali disease" or "blind staggers" is common. This disease is due to selenium poisoning. The soil contains selenium which is absorbed by plants growing on it. These plants are then toxic to animals eating them.

Boron has been shown to be essential for the development of many plants. No function is known for the other elements mentioned, but the future may show that they also have some function in nutrition.

Organic Foods

Milk. Of our common foods two may be considered nearly ideal, namely, milk and eggs. Milk is the food that nature has provided for young mammals, and eggs furnish the nutrients necessary for the embryonic development of chicks. The composition of the milk of different species of animals varies. Table 8 gives the approximate composition of various kinds of milk together with the time required to double the birth weight for each species. It is interesting to note that there is a definite relationship between the time required to double the birth weight and composition of the milk. As the time decreases the percentage of protein and ash in the milk increases. This would be expected since protein is needed to build body tissue and mineral matter is essential for bone development. With lactose the relationship is reversed; slow-growing animals have more sugar in their milk than those which grow rapidly because the former require energy-containing foods rather than tissue-building foods.

TABLE 8

COMPOSITION OF VARIOUS KINDS OF MILK

Kind of Milk	Time Required to Double Birth Weight (days)	Water (per cent)	Fat (per cent)	Lactose (per cent)	Protein (per cent)	Ash (per cent)
Human	180	87.4	4.0	7.0	1.4	0.2
Mare	60	90.6	1.1	5.9	1.8	0.4
Cow	47	87.1	3.9	4.9	3.4	0.7
Goat	22	87.0	4.2	4.8	3.7	0.8

Milk for Infant Feeding. From Table 8 it will be noted that the milk of the mare resembles much more closely the composition of human milk than does that of the cow. For this reason mare's milk is a better substitute than cow's milk for human milk.

In comparing the composition of cow's and human milk it will be noted that human milk is much richer in lactose and poorer in protein and ash than cow's milk. When cow's milk is used in infant feeding, it is customary to dilute it to bring the protein and ash content down to the proper amounts and then to add sugar to supply the energy lost by lowering the lactose and fat content. It appears that lactose should be the sugar added, but it is more common to add other carbohydrates, such as Dextri-maltose which is a partially hydrolyzed starch preparation. Corn syrup, which contains several of the degradation products of corn starch, has gained wide use in infant feeding. Cane sugar gives good results, its advantage being that it is readily available in a pure form and at a low cost. At the present time several milk powders are on the market which, when dissolved in the proper amount of water, give a solution very similar to human milk in composition. These have been very satisfactory as substitutes for human milk.

Soft-Curd Milk. One of the reasons why infants fail to do well on cow's milk is that the curds which form from it are large and hard and are not easily digested. Often large white curds appear in the feces of infants fed on cow's milk. Much work has been done on methods of softening the curd of milk. It has been found that some cows give milk which forms a softer curd than others. Attempts have been made to obtain for infants milk from only those cows which produce a soft-curd milk. As boiling softens the curd of milk, this practice is common in infant feeding. It should be pointed out in this connection that boiling lowers the vitamin potency of milk; however, this is not a serious handicap, since vitamins may be supplied from other sources. Another way to soften the curd is to add an acid, such as lactic acid, to the milk mixture. If this is done with stirring, the casein precipitates in fine particles, which are easily digested. At the present time many physicians are recommending evaporated milk for infant feeding, since its curd is softer than that of fresh milk. Still another satisfactory product for infants is homogenized milk. Homogenized milk has been passed through fine openings under pressure in order to break up the fat globules into very fine particles. In fact, the fat particles are so small that they will not rise to the surface to form a layer of cream. Homogenization also lowers the curd tension of milk.

Colostrum. The first milk produced in a lactation period is called colostrum. It is quite different from ordinary milk in composition. Ordinary cow's milk has about 13 per cent of total solids, whereas colostrum has about 25 per cent. The main difference is in the protein fraction. Colostrum contains more than 13 per cent of albumin and globulin.

The percentage of total solids in human colostrum and milk is about the same. Colostrum contains more protein and ash and less lactose and fat than milk.

It is quite generally believed that it is important for an infant to receive its mother's colostrum, which has a laxative effect helpful in cleaning out the digestive tract of the newborn. Colostrum is rich in globulin, similar to that found in the blood, which is thought to be responsible for immunity to certain contagious diseases. The blood of infants is low in this globulin. The high concentration of globulin in colostrum may aid an infant in building up his blood globulin and consequently his resistance to disease. It is well known that infants are immune to many contagious diseases. It has been suggested that colostrum may be an important factor in developing such immunity.

Colostrum is much richer in vitamin A than milk secreted later in a lactation period. It may be that the extra supply of this vitamin received in the first few days of life is important for the well-being of the infant. Vitamins C and D are slightly higher in colostrum than in milk.

Proteins of Milk. The main protein in milk is **casein,** which is a phosphoprotein. When milk sours, the lactose is converted into lactic acid by bacterial action. At *p*H 4.7 the isoelectric point of casein is reached, the casein precipitates, and the milk is said to curdle. The precipitated casein is used frequently as a food and is known as cottage cheese. In the commercial manufacture of cheese the casein is precipitated by means of **rennin.** Rennin is an enzyme, found in the stomach of calves, which converts casein into **paracasein.** Paracasein in the presence of calcium salts forms **calcium paracaseinate,** which is insoluble and precipitates. This precipitated calcium paracaseinate, along with most of the fat in the milk, is removed, compressed, and allowed to ferment or ripen to form our common cheese. The kind and flavor of the cheese depend upon the type of microorganism present during the fermentation process. It should be emphasized that when casein is precipitated by acid the whole casein molecule is precipitated; when rennin is used the casein is precipitated as calcium paracaseinate.

Milk contains two other proteins besides casein, namely, **lactalbumin** and **lactoglobulin.** Both are of high nutritional value. Human milk contains more lactalbumin than cow's milk. This is thought to be a factor in soft-curd formation when human milk is fed to infants.

Lactose. The carbohydrate in milk is lactose, a disaccharide composed of glucose and galactose. Since galactose is found in foods, other than milk, only in small amounts, it is evident that there must be a means of synthesizing galactose in the body. This synthesis is thought to occur in the mammary gland from glucose. Here also it is combined with glucose to form lactose.

Galactose is found in the brain and nervous tissue as a constituent of glycolipids. The lactose in milk may be an important source of galactose for the synthesis of these compounds.

Lipids in Milk. Most of the lipid material in milk is fat. Small amounts of free fatty acids, phospholipids, and cholesterol are also present. In the fat of cow's milk about 10 per cent of the fatty acids present are volatile, which indicates that they have less than ten carbon atoms in the molecule. Human-milk fat contains few volatile fatty acids.

From the commercial standpoint the fat of milk is a very important product. It is sold as cream and butter and is an important constituent of cheese. Milk is often sold on the basis of its fat content.

A simple procedure for determining the quantity of fat in milk is the **Babcock method** in which measured amounts of milk and concentrated H_2SO_4 are placed in a special flask with a narrow graduated neck. The acid dissolves all the milk except the fat which remains as a liquid layer at the surface. The heat generated by the acid when added to the milk keeps the fat liquid. Water is then added in sufficient amount to bring the fat into the graduated neck of the flask. After centrifugation the length of the column of fat in the neck of the flask is read. The calibrations are such that percentage of fat in the sample of milk analyzed is read directly.

Minerals in Milk. Milk contains a variety of mineral salts, in fact, with the exception of iron, all that are found in the body of the newborn animal. Iron must be supplied in other foods or nutritional anemia develops. Milk is especially rich in calcium, magnesium, sodium, potassium, phosphorus, and chlorine. Calcium and phosphorus are important for bone development. Milk contains four times as much potassium as sodium.

Pasteurization. Milk in the mammary gland is sterile, but, as we get it, it always contains microorganisms. Normally the microorganisms present are not harmful, but, since milk is such an excellent medium for bacterial growth, great care must be taken to prevent contamination with pathogenic organisms. Many serious epidemics of such diseases as typhoid fever have been traced to a contaminated milk supply. In order to prevent the possibility of spreading disease through milk it is customary to **pasteurize** it, usually by heating the milk to 145°F. and holding it at that temperature for 30 minutes. This method of heating has been found sufficient to destroy all pathogenic organisms, but many of the harmless organisms survive this treatment. The presence of lactic acid bacteria in milk is desirable because, if conditions are right for bacterial growth, these bacteria will multiply and sour the milk. Many undesirable organisms will not grow in sour milk; thus nature provides a means of protecting this important foodstuff. In Pennsylvania a special grade of pasteurized milk, known as **Grade A pasteurized milk,** is recognized. In order to be sold as this grade the milk must be produced from disease-free animals under sanitary conditions and, when sold, must not have a bacterial count above 30,000 per milliliter.

Certified Milk. It has been shown that the vitamin potency of milk is reduced by the process of pasteurization, and hence some persons consider it desirable to use raw milk. In order that people may feel safe in feeding children raw milk, health authorities have allowed certain producers to sell their milk as certified. Certified milk is produced from disease-free herds which are kept under rigid sanitary conditions. All employees must pass a physical examination to insure that they are not carriers of disease. The milk must be handled in a sanitary manner and must not contain more than 10,000 bacteria per milliliter. Although certified milk should be free from disease-producing organisms, there is no assurance that it is. Some persons believe that even certified milk should be pasteurized, and many producers are placing on the market pasteurized certified milk.

Milk as a Food. Besides containing food constituents necessary for body building and energy production, milk also supplies the vitamins which are so important for health. The yellowish color of cream is due mainly to carotene, a pigment closely related to vitamin A. Summer milk is more highly pigmented than winter milk and also has higher vitamin A activity. The greenish fluorescence of milk whey is due to the presence of riboflavin, a pigment possessing vitamin activity. Riboflavin is one of the vitamins of the vitamin B complex. Milk does not furnish all the vitamins in sufficient quantities for the needs of children. It is often deficient in vitamins C and D. These deficiencies are counteracted by feeding orange juice to supply vitamin C and cod-liver oil to supply vitamin D.

Besides being deficient in certain vitamins, milk does not contain sufficient iron for body needs. As mentioned above, if iron is not supplied to an animal on a milk diet, nutritional anemia develops. At birth a child has a generous supply of iron stored in his red blood cells. Blood studies on young children have shown that at birth the hemoglobin content and red-cell count are high. These gradually decrease with age; if iron is not supplied in the diet, anemia develops. Perhaps the best food for supplying iron in an infant's diet is the yolk of egg.

The value of milk in the diet has been strikingly demonstrated by Sherman, who fed two groups of rats on different diets, both of which were adequate from the standpoint of nutrients present. The difference between them was that one contained an abundance of milk and the other did not. The group receiving the abundance of milk lived on the average about 10 per cent longer than those on the other diet. Applying this knowledge to human nutrition, Sherman believes that the average span of human life would be lengthened by a more liberal intake of milk.

Eggs. Since eggs contain the necessary food requirements for the embryonic development of the chick and since birds have in general the same food requirements as mammals, eggs rank with milk in their value as a food. Since chicks, at hatching, must have an adequate blood supply, the egg must contain sufficient iron for the synthesis of the blood. This iron is found mainly in the yolk. In milk the calcium is present in the form of inorganic salts and also in combination with casein. In eggs much of the calcium is present in the shell, which is dissolved and absorbed as incubation proceeds. Calcium is present also in organic combination and as salts in the egg itself. In milk, phosphorus is present as inorganic phosphates and in the phosphoprotein casein. In eggs, phosphorus is found in the form of inorganic phosphates in solution and in the shell. In the yolk a phosphoprotein called **vitellin** corresponds to the casein of milk. In the yolk there is also much lecithin, a phospholipid. Milk contains very little phospholipid. In milk there is around 4 per cent of butterfat; in egg yolk an oil is present, called egg oil. The sugar in eggs, a trisaccharide made up of two molecules of mannose and one of glucosamine, is present in small amounts.

An egg has two parts, the yolk and the white. The yolk is the real egg cell, the greater part of which is the food supply for the developing embryo, and not living protoplasm. Around the yolk is the white, which is a secretion of the oviduct.

The yolk of the egg contains large amounts of oil, considerable lecithin, and some cholesterol. The main protein in the yolk is vitellin. The yellow substance of the yolk is mainly **xanthophyll,** a pigment found in green leaves. It is interesting to note that in the summer, when chickens have access to greens, eggs are highly pigmented, but in the winter much of the pigment is lacking. The ash of the yolk is composed mainly of sodium, potassium, calcium, magnesium, iron, phosphorus, and silicon.

The white of eggs is often spoken of as egg albumin. Egg white contains an albumin, but this is not the only protein present. More than 6 per cent of the protein present is a globulin. There is a considerable amount of glycoprotein in egg white which, along with a small amount of carbohydrate, is responsible for the positive Molisch test given by egg white. Egg white contains a considerable amount of sulfur. As eggs age, this sulfur is liberated in the form of sulfides, which react with the iron of the yolk to form iron sulfide. This reaction is responsible for the blackening on the surface of the yolks of cooked eggs. The sulfides are also responsible for the blackening of silverware used with eggs; this discoloration is silver sulfide. The ash of the white is composed mainly of sodium, potassium, and chloride, together with small amounts of calcium, magnesium, and phosphate.

Eggs are a fine source of vitamins; in fact, they are one of the few natural foods in which the vitamin distribution is fairly adequate.

Meat. In the adult diet meat is one of the main sources of protein. Since proteins are used by the body for building tissue, and since their value for this purpose depends upon the kinds of amino acids present, it appears that meat is a very valuable protein food. Since meat is the body tissue of animals, it should give on digestion a mixture of amino acids especially suited for building the body tissues of the animal eating it. Besides protein, meat contains a considerable quantity of fat, which is valuable for heat and energy production. Some meats, such as bacon, are largely fat. Meats contain a small amount of glycogen, which serves as a carbohydrate food. Liver is especially rich in glycogen. It has been demonstrated that a person can live on foods of animal origin alone.

Cereal Foods. Among the foods made from cereals are bread and breakfast foods. They are classed as carbohydrate foods, although they contain around 10 per cent of protein. They contain very little fat. They are used mainly to supply energy but also contribute to the protein requirements of the body to a considerable extent.

Vegetables and Fruits. Vegetables vary considerably in their nutritional value. Some, like potatoes, which are very high in starch, are important as sources of carbohydrate in the diet. Others, such as celery and lettuce, are low in calorific properties. Perhaps one of the most useful properties of vegetables in the diet is that they give bulk to the food. Many of them contain much cellulose, which is indigestible and therefore passes through the intestinal tract, giving bulk to the feces. Vegetables contain carbohydrates, fats, proteins, and mineral salts which are utilized by the body. They are also very valuable for their vitamin content. As a rule vitamin potency is correlated with pigmentation. Thus the green leaves of lettuce are better from this standpoint than the crisp, colorless center of the head. Fruits are important because they contain nutrients and because they stimulate the appetite. Citrus fruits are particularly valuable as a source of **vitamin C.** Nuts are extremely nutritious. They are especially rich in fat, and some, like the peanut, contain large amounts of carbohydrate.

An Adequate Diet

In this chapter we have considered very briefly some of the important aspects of foods and nutrition. In conclusion it is well to consider the chief factors in an adequate diet. Proteins must be eaten in such amounts and quality as to supply the amino acids necessary for the growth and repair of body tissues. To these proteins must be added sufficient carbohydrate and fat so that the total food consumed will give the required number of calories. An average adult requires about 3000 Calories per day, which would be supplied by 70 grams of protein, 80 grams of fat, and 500 grams of carbohydrate. In meeting these requirements foods should be selected which will supply the proper minerals and vitamins. This can best be done by including in the diet generous quantities of milk and leafy vegetables.

REVIEW QUESTIONS

1. Define food.
2. Discuss the importance of water to the animal body.
3. What is meant by metabolic water? Bound water?
4. Name the inorganic elements found in the body and indicate the importance of each.
5. Discuss the absorption, storage, and utilization of iron in the body.
6. Compare the composition of human, cow's, and mare's milk.
7. How is cow's milk modified for infant feeding?
8. Name several methods of producing soft-curd milk.
9. What is colostrum? Why is it important in infant nutrition?
10. Name the important proteins in milk.
11. Indicate two methods of precipitating the casein of milk.
12. Give the chemistry involved in the precipitation of casein by rennin.
13. Name the sugar of milk. What may be its special value in nutrition?
14. In what respect does the fat of cow's milk differ from that of human milk?
15. Describe the Babcock method for determining fat in milk.
16. What is pasteurized milk? Why is milk pasteurized?
17. What is Grade A pasteurized milk?
18. What is certified milk?
19. Discuss milk as a food. In what respect is milk deficient as a food?
20. Compare the composition of milk and eggs.
21. Compare the iron content of milk and eggs.
22. Name the proteins of eggs.
23. What is the main pigment in egg yolk?
24. Discuss meat as a food.
25. Discuss cereals, vegetables, and fruits as foods.
26. What factors must be considered in selecting an adequate diet?

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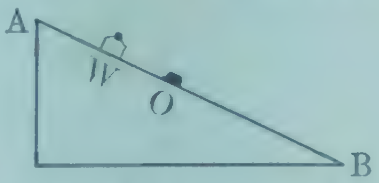
7

Enzymes

One of the first experiments performed by a beginner in chemistry is the preparation of oxygen. If $KClO_3$ is heated to a high temperature in a test tube, oxygen is given off at a moderate rate. However, if MnO_2 is added to the $KClO_3$ before heating, oxygen is given off at a lower temperature and much more rapidly. Finally, after all the oxygen has been given off, KCl and MnO_2 remain in the test tube. The MnO_2 in some manner difficult to explain has hastened the decomposition of the $KClO_3$. Such a substance, which alters the speed of a reaction and remains, in the end, unchanged, is called a **catalyst.**

Many of the reactions which take place in protoplasm are very complicated. A great number have never been duplicated in the laboratory, and, of those which have, most have been carried out with difficulty. Since in protoplasm reactions occur with apparent ease, it is evident that catalysts must play an important role. These catalysts which are produced by living organisms have been given the name **enzymes.**

To explain in a simple way how enzymes and catalysts work the following analogy is useful. Let us imagine an inclined plane, *AB*, of such a slope that a weight, *W*, placed at *A* at 6 A.M. will move slowly down the plane and will reach the bottom, *B*, at 6 P.M. Now let us suppose



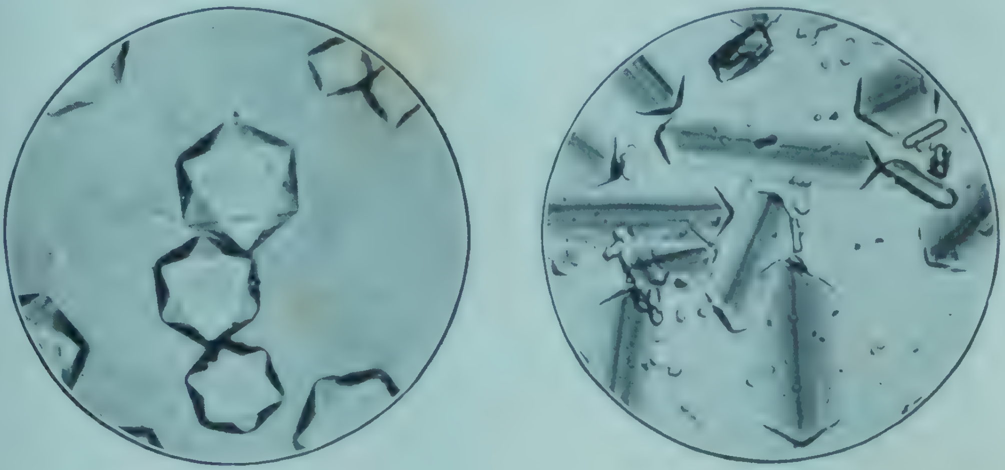
that a drop of oil is placed at *O*, a point which the weight will reach at 11 A.M. At a few moments past eleven the weight will reach the bottom of the inclined plane. The drop of oil has hastened the progress of the weight and has not been used up in the process. In other words, the drop of oil has catalyzed the physical reaction involved. In the diagram it should be noted that a drop of oil at *O* would be of no assistance to the weight if the weight did not move slowly by itself. In other words, the oil must get under the weight in order to lubricate it. Thus, to apply this analogy to enzymes, it must be assumed that reactions catalyzed by enzymes are proceeding slowly by themselves. Whether enzymes can initiate chemical reactions or can only hasten those already in progress is a debatable question. For practical purposes it may be assumed that enzymes can initiate a chemical reaction.

It is obvious that this analogy does not explain the chemical mechanism involved in enzyme action. Although information is not conclusive, some light will be thrown on this subject later in the chapter.

Exo- and Endoenzymes. In the early literature enzymes were spoken of as **ferments,** and it was thought that there were two types of ferments. Those like **yeast,** in which the living cell was considered essential, were called **organized ferments;** those like **pepsin,** the protein-hydrolyzing enzyme in the stomach, which contained no living organisms, were called **unorganized ferments.** In 1897 Buchner ground yeast cells with sand and pressed out from this mixture a juice which fermented sugar just as yeast itself did. In this way he proved that the living yeast cell is not essential for the fermentation of sugar. In other words, there is no essential difference between organized and unorganized ferments. In the one, the enzyme is in a microscopic organism and can be removed after destroying the organism. In the other, the enzyme is in a large organism from which it may be separated without destroying the organism. When Buchner's method is applied to other so-called organized ferments, it is possible in most of them to separate the enzyme responsible for the fermentation, which works without the presence of the living organism. However, it should be mentioned that some enzymes, up to the present time, have not been separated from the organism producing them. Enzymes which normally act within the cell are called **endoenzymes,** and those which normally work after they have been secreted by the cell are called **exoenzymes.** The enzymes of the digestive tract are good examples of exoenzymes.

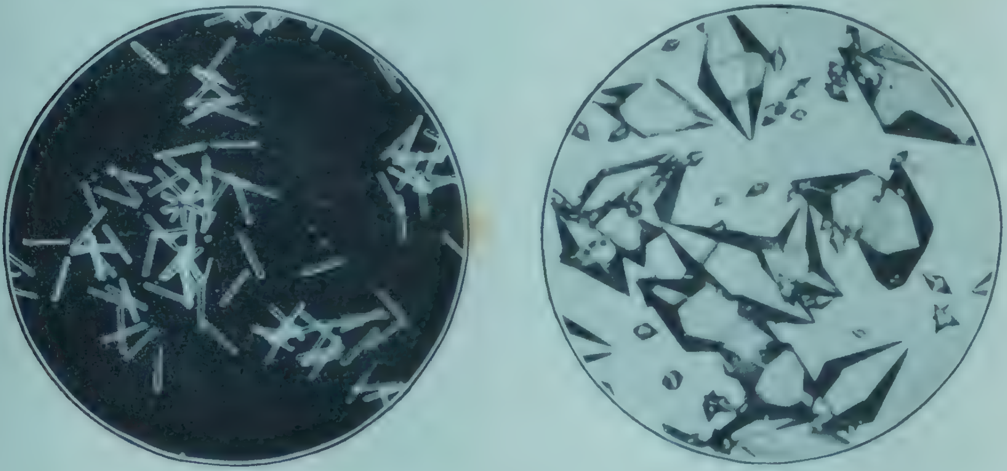
Nomenclature. The substance upon which an enzyme acts is called the **substrate.** A common system of naming enzymes is to add the ending **-ase** to the root of the substrate. For example, the enzyme that hydrolyzes sucrose is called **sucrase.** Sometimes an enzyme is named by adding the ending **-ase** to a word descriptive of the reaction which it catalyzes. For example, an oxidase enzyme catalyzes an oxidation. However, many of the enzymes were known long before these systems of naming were suggested. Hence many of the common enzymes are usually referred to by their old names. A good example is pepsin, the protein-hydrolyzing enzyme of the stomach. Today it would be perfectly proper to speak of pepsin as the gastric protease.

Chemical Nature of Enzymes. Concerning the chemical nature of enzymes it may be said that they all contain protein. Some are simple proteins and others consist of a protein combined or associated with a nonprotein component. An example of an enzyme that is a simple protein is urease, which converts urea into carbon dioxide and ammonia. It is a globulin and was first prepared in crystalline form by J. B. Sumner in 1926. In 1946 Sumner received the Nobel prize for being the first to prepare a pure enzyme. Since 1926 many enzymes have been prepared in crystalline form which have turned out to be pure proteins.



Urease (Sumner)

Beef catalase (Sumner)



Trypsin (Kunitz and Northrop)

Pepsin (Northrup)

Fig. 17. Enzyme crystals. Courtesy of Drs. J. B. Sumner and John H. Northrop.

Many enzymes, notably those associated with oxidations and reductions, appear to be made up of two parts; one is protein, and the other is a simple organic compound. The two parts appear to be in loose chemical combination, and the complete enzyme is called a **holoenzyme.** When such an enzyme is submitted to dialysis, the protein fraction does not pass through the membrane, but the simpler organic compound does. The protein part of the enzyme has been called an **apoenzyme,** and the part which passes through the membrane has been called the **coenzyme** or the **prosthetic group** of the enzyme. Neither the apoenzyme nor the coenzyme is active by itself, but when they are mixed together the active enzyme is regenerated. Apoenzymes, being protein in nature, are destroyed by heat, probably because of denaturation. Coenzymes are not destroyed by heat.

Activators and Kinases. Many enzymes are stimulated by the presence of simple molecules or ions. The amylases of the saliva and of the pancreatic juice work better in the presence of the chloride ion, and the bile salts stimulate the activity of pancreatic lipase. Several bivalent ions such as magnesium, manganese, cobalt, and nickel are known to stimulate the action of certain enzymes. These simple enzyme stimulators are called **activators.**

Some enzymes, as produced by the cell, are inactive and require another substance to activate them. The inactive form of an enzyme is called a **zymogen,** or **proenzyme,** and the substance that converts it into an active form is called a **kinase.** In the pancreatic juice there is a proenzyme, called trypsinogen, which is converted into active trypsin by a kinase, called enterokinase, found in the intestinal juice. Enterokinase is a protein; it will not diffuse through a membrane and its activity is destroyed by heat. Some persons consider enterokinase to be an enzyme which converts trypsinogen into trypsin.

Inhibition of Enzymes. We have mentioned substances which act as stimulators for enzyme action. There are also substances which act as inhibitors to enzyme action. Salts of heavy metals, such as mercury, when added to enzyme solutions, inhibit their action either by precipitating the enzyme or by uniting with the active groups in the enzyme molecule.

In the body there are important inhibitors of enzyme action called **antienzymes.** It is thought that the reason for our not digesting our own stomachs and intestines, which are protein and should be hydrolyzed by proteases, is the presence of antienzymes in the living tissues of the stomach and intestinal lining. Tapeworms are not digested in the digestive tract of the host because of antienzymes which they contain. If repeated doses of rennin, a milk-curdling enzyme, are injected into a cow, the milk from that cow will no longer be curdled by rennin. It is believed that an antienzyme, which inhibits the action of rennin, is produced by the cow and secreted in the milk. If the enzyme urease is injected into a chicken, the blood of that chicken will contain antiurease.

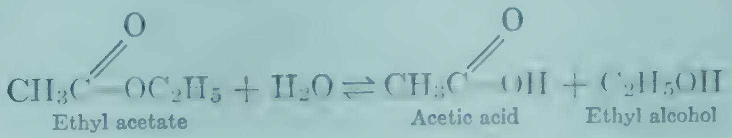
Mechanism of Enzyme Action. Little can be said with certainty about how enzymes act. However, it is believed that they attach themselves to the substrate either chemically or by adsorption, rendering it more unstable. As the substrate breaks down, the enzyme is liberated and attaches itself to more substrate, and the process is repeated. Thus a small amount of enzyme reacts with a large amount of substrate. The colloidal nature of enzymes makes available large surfaces for the adsorption of the substrate.

It is likely that only certain groups in the enzyme molecule are involved in its attachment to the substrate. This explanation would account for the fact that not all colloidal substances have enzyme activity. In other words, of two proteins very similar in properties, one may be an enzyme and the other not.

Specificity of Enzymes. Enzymes are very specific in their action. Some, such as urease, attack a specific compound and no other. Some will attack many different molecules having similar chemical structures. For example, maltase hydrolyzes α-glycosides and emulsin β-glycosides. Others attack large groups of similar compounds. For example, pepsin will hydrolyze all soluble native proteins.

Emil Fischer has explained enzyme specificity by assuming that an enzyme must fit its substrate as a key fits a lock. Some enzymes are specific keys which fit only one lock, whereas other enzymes are master keys which fit many locks.

Reversibility of Enzyme Action. Most of the chemical reactions taking place in protoplasm are reversible; that is, the reaction may proceed in either direction, depending upon conditions. A reversible reaction does not go to completion unless the end products of the reaction are removed. Under normal conditions the reaction proceeds until an equilibrium is established. When ethyl acetate is allowed to stand in contact with water, it slowly hydrolyzes to form acetic acid and ethyl alcohol.



$$\underset{\text{Ethyl acetate}}{CH_3\overset{O}{\overset{//}{C}}-OC_2H_5} + H_2O \rightleftharpoons \underset{\text{Acetic acid}}{CH_3\overset{O}{\overset{//}{C}}-OH} + \underset{\text{Ethyl alcohol}}{C_2H_5OH}$$

If ethyl alcohol and acetic acid are mixed, they will slowly combine to form ethyl acetate and water. According to the law of mass action, the speed of a reaction is proportional to the concentration of the reacting substances. In the foregoing equation, if one started with ethyl acetate and water, the initial concentration of these substances would, of course, be high, and the reaction would proceed from left to right. As soon as acetic acid and ethyl alcohol were formed, they would react with each other to reverse the original reaction. At first this reaction would be very slow because the concentration of acetic acid and ethyl alcohol would be low. However, as the concentrations of these two substances increased, the speed of the reaction from right to left would increase, until finally it would equal the speed of the reaction in the opposite direction. At this point equilibrium would be established. If an end product of either reaction were continuously removed, the reaction would go to completion in the direction of the removed end product. For example, if after equilibrium had been reached, something were added that would remove water, the reaction would proceed until all the acetic acid and ethyl alcohol had united to form ethyl acetate.

Enzymes added to a reacting mixture do not cause the reaction to go to completion but simply hasten the establishment of an equilibrium. The same enzyme will hasten the equilibrium in either direction. Thus if to an ethyl acetate-water mixture an ester-hydrolyzing enzyme is added, the ester hydrolyzes very rapidly to form the equilibrium mixture. If the enzyme is added to an alcohol-acetic acid mixture, the same equilibrium mixture is rapidly formed. What has just been said is an example of the **reversibility of enzyme action.**

When protein is eaten, enzymes in the digestive tract hydrolyze it to form amino acids. These amino acids, which are the end products of the reaction, are removed by being absorbed into the blood stream. In this way it is possible for the reaction to go to completion. In the body tissues possibly the same enzymes are responsible for the synthesis of these amino acids into tissue proteins.

It should be pointed out that not all of the reactions occurring in protoplasm are reversible and that in certain cases reversible reactions require different enzymes for catalyzing the reaction in opposite directions.

Conditions for Enzyme Activity. The rate of enzyme activity is influenced by many factors. Perhaps the most important are **temperature, hydrogen-ion concentration of the medium,** and **time.** Other factors which may be mentioned are concentration of the enzyme, substrate, and end products, electrolytes present, and light. Thus it is obviously difficult to state what the optimum condition is with respect to any one of these factors, since it may differ with a variation of the other factors.

It is a generally known fact that the speed of a chemical reaction increases as the temperature rises. According to Van't Hoff's rule, the speed of a chemical reaction doubles or trebles for every 10°C. rise in temperature. The degree to which a reaction is speeded up by a 10°C. rise in temperature is known as the **temperature coefficient** of the reaction. In reactions catalyzed by enzymes this rule holds to a certain point, at which there is a rapid falling off of activity. This decrease is explained on the basis of inactivation of the enzyme by heat. In other words, the action of the enzyme may be hastened by higher temperatures, but a point is soon reached where the destruction of the enzyme is the predominating factor and there is a slowing up of the rate of the reaction caused by the reduction of amount of enzyme present.

Since the speed of a chemical reaction is measured by the amount of chemical change produced in a given time, it is apparent that the time factor enters into all enzyme studies. In determining the temperature at which an enzyme reaction is proceeding most rapidly, the time allowed for the enzyme to act must be considered. An enzyme may catalyze a reaction very rapidly for a short period, but over a long period of time the enzyme might be destroyed by the high temperature and the amount of substrate changed would be very little in comparison to that changed at a lower temperature. Thus it is evident that a statement that an enzyme acts best at a certain temperature or *p*H applies only for a given set of conditions and may not be true if these conditions are altered.

The speed at which an enzyme reaction proceeds is greatly influenced by temperature. The temperature at which the reaction goes on most rapidly is said to be the **optimum temperature** for that enzyme. The optimum temperature for most of the body enzymes under ordinary conditions is around 37° to 45 C. Some enzymes have their optimum at a higher temperature. It may be said that generally low temperatures do not destroy an enzyme. Its activity returns upon heating to optimum temperature. Temperatures of 60° to 80°C., however, permanently destroy most enzymes. The majority of enzymes are inactive at 0°C. Exceptions to this rule are fat-hydrolyzing enzymes, which have been found to be active at −15°C. Thus fat-containing foods may deteriorate when stored below 0 C. unless care is taken to destroy lipases before storage.

Enzyme action is also influenced by the reaction, or *p*H, of the medium. Each enzyme works best at a rather definite *p*H, which is spoken of as the optimum. The optimum varies widely for different enzymes. Pepsin is most active at a *p*H of 1.9, whereas trypsin, the protein-splitting enzyme of the pancreatic juice, works best at a *p*H of 8.1. No enzyme works beyond the *p*H range of 1 to 13.

Classification of Enzymes. The accompanying classification lists some of the more important enzymes, as well as the occurrence of each, the substrate upon which it acts, and the end products which it produces.

CLASSIFICATION OF ENZYMES

Name	Occurrence	Substrate	End Products
I. Hydrolases			
A. Carbohydrases		Carbohydrates	Hydrolytic products
1. Amylases		Starch and glycogen	Maltose
a. α-Amylase	Plants and animals	Starch and glycogen	Dextrins
b. β-Amylase	Plants and animals	Starch and glycogen	Maltose
2. Sucrase	Intestinal juice	Sucrose	Glucose and fructose
3. Lactase	Intestinal juice	Lactose	Glucose and galactose
4. Maltase	Intestinal juice	Maltose and α-glycosides	Glucose, other sugars, etc.
5. Emulsin	Plants	β-Glycosides	Sugar, etc.
B. Esterases		Esters	Acids and alcohols
1. Lipases		Fats	Fatty acids and glycerol
a. Pancreatic lipase	Pancreatic juice	Fats	Fatty acids and glycerol
b. Vegetable lipase	Castor bean	Fats	Fatty acids and glycerol
2. Phosphatase	Tissues	Organic phosphates	H_3PO_4, etc.
3. Choline esterase	Brain and muscle	Acetylcholine	Choline and acetic acid
C. Nucleases		Nucleic acid and derivatives	Hydrolytic products
1. Polynucleotidase	Intestinal juice	Nucleic acid	Nucleotides
2. Nucleotidase	Intestinal juice	Nucleotides	Nucleosides and H_3PO_4
3. Nucleosidase	Tissues	Nucleosides	Sugar and purines or pyrimidines
D. Amidases		Amides	Hydrolytic products
1. Arginase	Liver	Arginine	Ornithine and urea
2. Urease	Soybeans and jack beans	Urea	CO_2 and NH_3

CLASSIFICATION OF ENZYMES (*Continued*)

Name	Occurrence	Substrate	End Products
E. Proteases		Proteins	Hydrolytic products
1. Pepsin	Gastric juice	Proteins	Proteoses and peptones
2. Trypsin	Pancreatic juice	Proteins	Proteoses, peptones, and polypeptides
3. Chymotrypsin	Pancreatic juice	Proteins	Proteoses, peptones, etc.
4. Rennin	Gastric juice	Casein	Paracasein
5. Cathepsin	Animal tissues	Proteins	Proteoses and peptones
6. Bromelin	Pineapples	Proteins	Proteoses and peptones
F. Peptidases		Peptides	Simpler peptides and amino acids
1. Aminopolypeptidase	Intestines	Polypeptides	Simpler peptides and amino acids
2. Carboxypolypeptidase	Pancreas	Polypeptides	Simpler peptides and amino acids
3. Prolinase	Intestines	Polypeptides containing proline	Simpler peptides and proline
4. Dipeptidase	Intestines	Dipeptides	Amino acids
II. Oxidases			
A. Dehydrogenases	Tissues	Organic compounds	Oxidation products
B. Catalase	Plant and animal tissues	H_2O_2	Molecular oxygen
C. Peroxidase	Plant and animal tissues	Organic peroxides	Nascent oxygen and oxidation products
D. Tyrosinase	Plant and animal tissues	Tyrosine	Black oxidation product
E. Uricase	Animal tissues	Uric acid	Allantoin
F. Ascorbic acid oxidase	Plants and animals	Ascorbic acid	Dehydroascorbic acid
III. Desmolases			
A. Decarboxylases			
1. Carboxylase	Yeast	α-Keto acids	CO_2 and aldehydes
2. Carbonic anhydrase	Red blood cells	H_2CO_3	CO_2 and H_2O
B. Transaminases			
1. Glutamic acid-alanine transaminase	Plants and animals	Glutamic and pyruvic acids	α-Ketoglutaric acid and alanine

The enzymes listed in the classification are divided into three main classes: the **hydrolases,** the **oxidases,** and the **desmolases.** The hydrolases break up complex molecules into simpler ones by the addition of molecules of water; the oxidases catalyze biological oxidations and reductions; and the desmolases break C—C, C—N, and other linkages in the substrate.

Hydrolases

The hydrolases are especially important because many of them are associated with the digestion of foods in the body. Most of the foods we eat are composed of large molecules which must be broken down into their simple components before they can be absorbed and utilized by the body. The breakdown of large food molecules in the digestive tract is a process of hydrolysis brought about by hydrolase enzymes.

Carbohydrases. Most of the carbohydrases are associated with the hydrolysis of di- and polysaccharides to simpler sugars. Those which hydrolyze starch and glycogen are called amylases. There are two amylases known as **α- and β-amylase.** α-Amylase hydrolyzes starch and glycogen to dextrins; β-amylase hydrolyzes starch, glycogen, and certain dextrins to maltose. The action of these enzymes in the hydrolysis of starch was discussed on p. 89. Salivary amylase (ptyalin), pancreatic amylase (amylopsin), and plant amlyases (diastase) are thought to be mixtures of α- and β-amylase.

The disaccharide-splitting enzymes **maltase, sucrase,** and **lactase** are important enzymes of the digestive tract because they hydrolyze the common disaccharides of the diet. Maltase hydrolyzes α-glycosides and is often used to identify them. Emulsin is included in the classification because it hydrolyzes β-glycosides and is used in their identification.

Esterases. The esterases hydrolyze organic esters to form the alcohols and acids of which the esters are composed. Those that hydrolyze fats are called **lipases.** Pancreatic lipase (steapsin) is responsible for the hydrolysis of most of the fat that we eat. Fat is hydrolyzed to form glycerol and fatty acids by lipase. An important vegetable lipase is found in the castor bean. Lipases are associated with the spoilage of fatty foods during storage.

Phosphatases hydrolyze organic phosphates and are important factors in carbohydrate and lipid metabolism and in bone formation. The phosphatase activity of the blood is often determined as an aid in the diagnosis of certain diseases.

Choline esterase is the enzyme that hydrolyzes acetylcholine to choline and acetic acid. This reaction is important because it occurs when muscles recover after being stimulated by nerves. (See p. 302.) Certain insecticides and nerve gases which have been suggested for use in chemical warfare owe their activity to the inhibition of choline esterase.

Nucleases. Three enzymes are involved in the hydrolysis of nucleic acid. **Polynucleotidase** hydrolyzes nucleic acid to nucleotides. **Nucleotidase** removes phosphoric acid from nucleotides to form nucleosides. Since nucleotidases remove phosphoric acid, they are phosphatases and are often classified as such. **Nucleosidases** hydrolyze nucleosides to sugars and purines or pyrimidines.

Amidases. The amidases hydrolyze amides. Two amidases are listed in the classification, namely, **arginase** and **urease.** Arginase is found in the liver and is an important factor in the synthesis of urea. For details of this synthesis see p. 272.

Urease, the enzyme which hydrolyzes urea to carbon dioxide and ammonia, is of special interest to the biochemist because it is used in the determination of urea in blood and urine. The samples to be analyzed are treated with the enzyme, and the ammonia formed is removed and determined by titration or by a colorimetric method involving the use of **Nessler's solution**, which gives a brown color with ammonium salts.

Proteases. The proteases hydrolyze proteins to simpler molecules such as proteoses, peptones, and polypeptides. They do not completely hydrolyze proteins to amino acids. **Pepsin**, found in the gastric juice, and **trypsin** and **chymotrypsin**, found in the pancreatic juice, initiate the digestion of protein foods in the body.

Rennin, found in the gastric juice of calves, is a proteolytic enzyme which acts on the casein of milk to form paracasein. In the presence of calcium salts, calcium paracaseinate is formed, which is insoluble and precipitates. Rennin is used commercially in the manufacture of cheese and in the home for preparing junket.

Cathepsins are proteolytic enzymes found in all animal tissues, especially in the liver, kidneys, and spleen, which are concerned with the autolysis or self-digestion of animal tissue after death. They are inactive at neutrality but become active at a *p*H of 4 to 5. During life body tissues are nearly neutral, but after death fermentations occur, resulting in the production of acids which change the *p*H to a point where cathepsins become active, with the result that tissue proteins are converted into proteoses and peptones. Cathepsins may be important factors in the aging of meat and in making it more tender.

Bromelin, a proteolytic enzyme found in pineapples, is of special interest to the housewife. In making fruit gelatin desserts fresh pineapples cannot be used because the bromelin present will hydrolyze the gelatin, forming products that will not gel. Cooked pineapple may be used because cooking destroys the enzyme.

Peptidases. Until recently an enzyme called erepsin was believed to be responsible for the hydrolysis of proteoses, peptones, and polypeptides to amino acids. The present conception of protein hydrolysis in the digestive tract is that **pepsin** hydrolyzes protein to proteoses and peptones, **trypsin** and **chymotrypsin** hydrolyze proteins or proteoses and peptones to polypeptides, and finally the polypeptides are hydrolyzed by a group of enzymes called **peptidases.** In other words, what was once known as erepsin has now been shown to be a mixture of peptidases.

A polypeptide is a chain consisting of several amino acids with a free amino group at one end and a free carboxyl group at the other. It is thought that, when polypeptides are hydrolyzed by peptidases, one amino acid is split off at a time. If the amino acid containing the free amino group is split off, the enzyme causing the hydrolysis is called **aminopolypeptidase.** If the amino acid containing the free carboxyl group is split off, the enzyme is called **carboxypolypeptidase.** If proline, an amino acid containing an imino group in place of an amino group, is on the end of the chain and is first split off, the enzyme is called **prolinase.** **Dipeptidases** hydrolyze dipeptides only.

Oxidases

Biological Oxidations and Reductions. The heat and mechanical energy of the body are a result of biological oxidations. Whenever there is an oxidation, there is always a reduction of the oxidizing agent, so that it is proper to consider oxidation and reduction together. Carbohydrates, fats, and proteins, the main sources of body energy, do not oxidize easily in the presence of atmospheric oxygen, but in the body they are readily oxidized; hence it is apparent that enzymes are involved in the process. These enzymes are called oxidases.

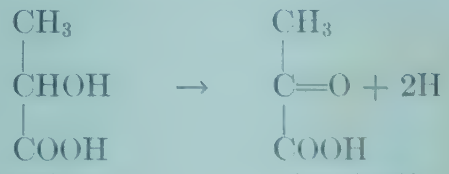
The chemistry involved in biological oxidations is not so simple as that just discussed in connection with the hydrolytic enzymes, and our knowledge on this subject is not complete. However, an attempt will be made to point out a few of the known facts regarding biological oxidation.

Oxidation is usually defined as the loss of electrons by the substance oxidized. Three ways in which this may occur in biological oxidations are: (1) by the addition of oxygen, (2) by the removal of hydrogen, or (3) by an increase in positive valency.

An example of the addition of oxygen is the oxidation of carbon to carbon dioxide.

$$C + O_2 \rightarrow CO_2$$

An example of the removal of hydrogen is the oxidation of lactic to pyruvic acid.



$$\underset{\text{Lactic acid}}{\begin{array}{c} CH_3 \\ | \\ CHOH \\ | \\ COOH \end{array}} \rightarrow \underset{\text{Pyruvic acid}}{\begin{array}{l} CH_3 \\ | \\ C{=}O + 2H \\ | \\ COOH \end{array}}$$

In this reaction each hydrogen atom removed contains an electron and so the lactic acid molecule has lost two electrons.

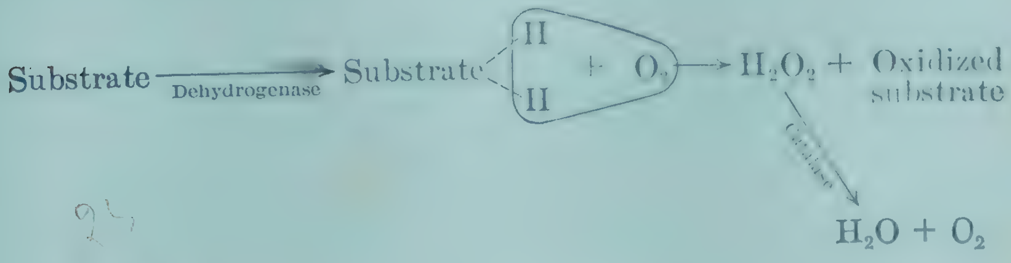
A good example of increase in positive valency is the oxidation of a ferrous to a ferric salt.

$$\underset{\text{Ferrous chloride}}{2FeCl_2} + Cl_2 \rightarrow \underset{\text{Ferric chloride}}{2FeCl_3}$$

In this case each ferrous ion, Fe^{++}, loses an electron to chlorine to become a ferric ion, Fe^{+++}.

Enzymes that catalyze the above types of reactions are called **oxidases.** When the reaction involves the addition of oxygen, it is thought that the enzyme converts molecular oxygen into atomic oxygen which is a better oxidizing agent. When the reaction involves the removal of hydrogen, it is thought that the enzyme activates hydrogen atoms of the substrate, rendering them readily removable by some easily reducible substance which must be present in order that the reaction proceed. Enzymes which activate hydrogen of the substrate are called **dehydrogenases.**

Perhaps the simplest form of biological oxidation is the one in which dehydrogenase activates two hydrogen atoms of the substrate, which unite with molecular oxygen to form H_2O_2 and the oxidized substrate. H_2O_2, which is toxic, is immediately converted into H_2O and O_2 by the enzyme catalase, which is present in all cells. The reactions involved may be expressed thus:



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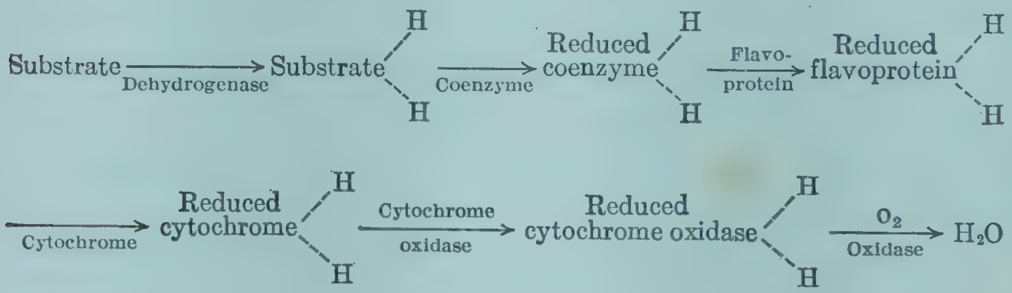
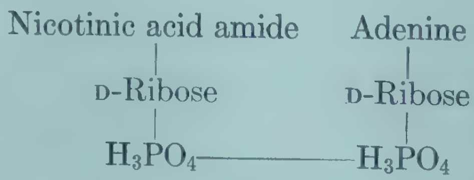
In this reaction the removal of activated hydrogen from the substrate is a fairly simple process. In most biological oxidations the process appears to be much more complicated, involving several intermediate steps. The first step, as in the reaction outlined, involves the activation of two hydrogen atoms of the substrate by dehydrogenase. The activated hydrogens are then transferred to an easily reducible substance called **coenzyme.** The reduced coenzyme next transfers its activated hydrogens to a **flavoprotein.** Next the reduced flavoprotein transfers its activated hydrogen to **cytochrome.** Next reduced cytochrome transfers its activated hydrogen to **cytochrome oxidase.** Finally reduced cytochrome oxidase is oxidized to cytochrome oxidase by molecular oxygen, a reaction in which water is formed. What has just been said may be represented diagrammatically as follows:

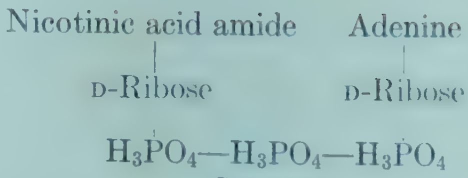

Substrate
Dehydrogenase
Substrate
H
H
Coenzyme
Reduced coenzyme
H
H
Flavo-protein
Reduced flavoprotein
H
H
Cytochrome
Reduced cytochrome
H
H
Cytochrome oxidase
Reduced cytochrome oxidase
H
H
O2
Oxidase
H2O

The intermediate compounds involved in this scheme are sometimes spoken of as hydrogen carriers, which may be looked upon as a bucket brigade transferring activated hydrogen from the substrate to the end product, H_2O. It should be noted that, as long as water is being formed, the reaction will continue to proceed from left to right. As each reduced intermediate compound passes on its activated hydrogen, it returns to the oxidized state, in which form it may accept more hydrogen and thus keep the reaction going.

Coenzyme I and II. Considerable is known concerning the chemical structure of the intermediate compounds. There are two coenzymes, known as coenzyme I and II. Both are dinucleotides but differ from each other in that coenzyme I contains two molecules of H_3PO_4, whereas coenzyme II contains three. Their structures may be represented thus:


Nicotinic acid amide
Adenine
D-Ribose
D-Ribose
H3PO4
H3PO4

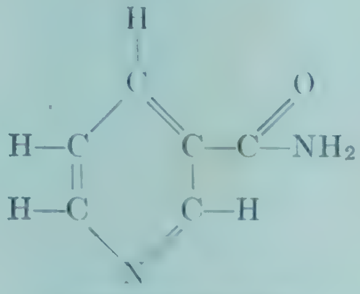
Coenzyme I



$$\begin{array}{ccccc} \text{Nicotinic acid amide} & & & & \text{Adenine} \\ | & & & & | \\ \text{D-Ribose} & & & & \text{D-Ribose} \\ | & & & & | \\ H_3PO_4 & — & H_3PO_4 & — & H_3PO_4 \end{array}$$

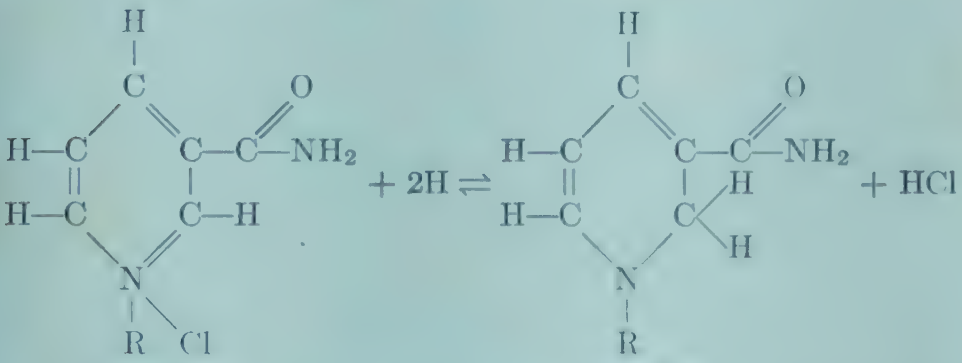
Coenzyme II

The active part of the coenzyme molecules which acts as a hydrogen acceptor appears to be nicotinic acid amide.



Nicotinic acid amide

In the coenzyme molecule the nicotinic acid amide is attached to D-ribose. In this compound the nitrogen is thought to be pentavalent. In the presence of activated hydrogen the nitrogen becomes trivalent, thus:



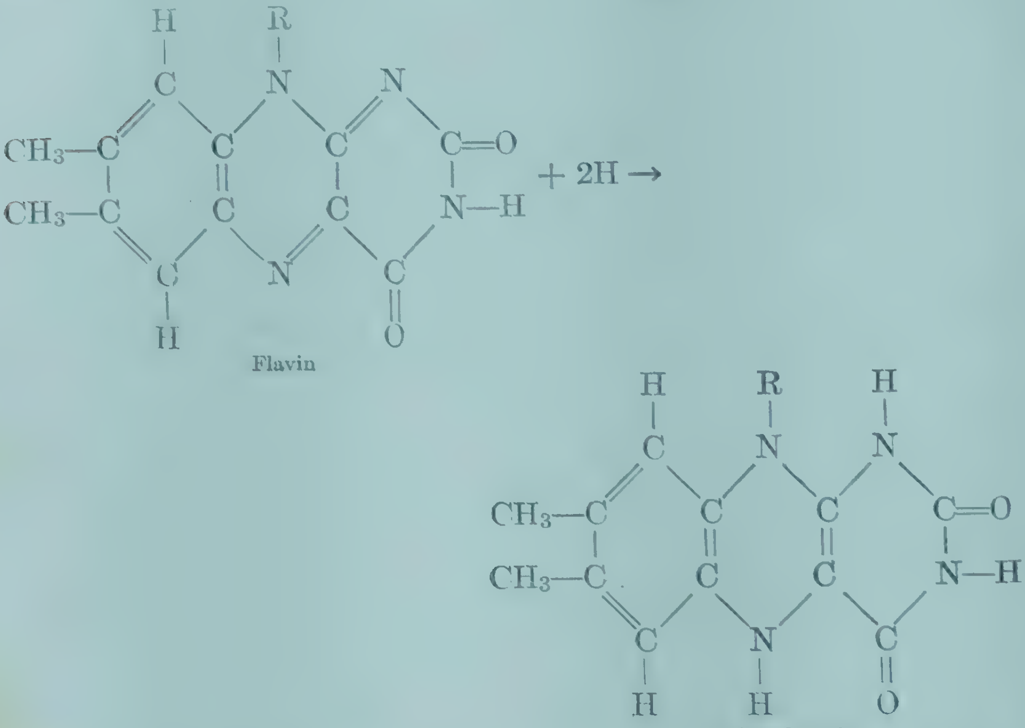
Oxidized form

Reduced form

Thus nicotinic acid amide acts as an oxidizing agent by removing hydrogen from the substrate. Nicotinic acid amide is essential in the diet and is one of the vitamins of the B complex, which will be discussed later.

It should be stressed that the coenzymes are not enzymes but only aid in the reactions started by dehydrogenases. Dehydrogenases only activate hydrogens of the substrate; coenzymes remove the activated hydrogens. Neither will cause an oxidation by itself.

Flavoprotein. As has been pointed out, reduced nicotinic acid amide passes its activated hydrogen on to a flavoprotein. A flavoprotein is composed of a protein combined with a nucleotide, which yields on hydrolysis flavin, D-ribose, and phosphoric acid. The active part of the molecule is the flavin, which accepts hydrogen as follows:



Flavin

Reduced flavin

It is noteworthy that a compound composed of D-ribose and flavin, called riboflavin, is a vitamin found in the vitamin B complex.

Cytochrome. From reduced flavoprotein hydrogen is passed on to cytochrome. There are three known cytochromes, referred to as a, b, and c; all three are involved in the transfer of hydrogen. The hydrogen first goes to cytochrome b, then to c, and finally to a. The cytochromes are conjugated proteins combined with an iron-containing compound similar to heme, the pigment in blood. The chemistry of heme will be discussed later. (See p. 316.) In oxidized cytochromes the iron has a valence of three, and in reduced cytochromes a valence of two. The iron is reduced by accepting an electron from the hydrogen which it receives. Thus the iron appears to be the active part of the molecule.

Cytochrome Oxidase. The final compound acting as a hydrogen acceptor in the foregoing scheme is cytochrome oxidase. Little is known about its chemical nature except that it contains iron. It appears that here, too, iron is the essential part of the molecule, acting in a manner similar to its action in cytochrome.

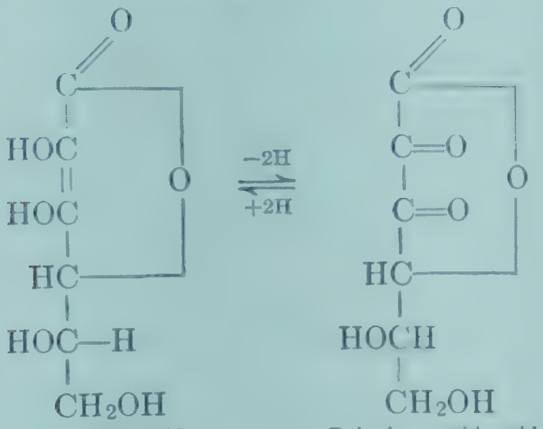
Glutathione. Besides the hydrogen acceptors mentioned there are two other compounds found in tissues that may play an important part in biological oxidations and reductions. These compounds are glutathione and ascorbic acid.

Glutathione is a tripeptide composed of **glycine, glutamic acid,** and **cysteine.** The important part of the molecule is the SH group of cysteine; therefore its formula is written RSH, where R represents the greater part of the molecule. It exists in two forms, reduced and oxidized.

$$\underset{\text{Reduced}}{2\text{RSH}} \rightleftharpoons \underset{\text{Oxidized}}{\text{RS—SR}} + 2\text{H}$$

In the oxidized form it could act as a hydrogen acceptor and thus as a coenzyme in a biological oxidation. However, it must be said that the exact role of glutathione in biological oxidations and reductions is not known.

Ascorbic Acid. Ascorbic acid, or vitamin C, is the other compound found in tissues that should be able to function as a coenzyme or carrier of hydrogen in biological oxidations. It may exist in two forms, the reduced form, called L-ascorbic acid, and the oxidized form, called dehydroascorbic acid. It is dehydroascorbic acid that should be able to act as a hydrogen acceptor in a biological oxidation. Perhaps its value as a vitamin is that it is essential for certain biological oxidations and reductions.



L-Ascorbic acid Dehydroascorbic acid

Catalase. Although catalase is classified as an oxidase, it does not cause biological oxidations. It acts on H_2O_2, converting it into H_2O and **molecular oxygen.** It is present in most living cells, and its function appears to be the removal of H_2O_2, which otherwise might accumulate during biological oxidation and which is toxic to living cells. Catalase is composed of a protein combined with heme. Sumner has prepared catalase in a crystalline form.

A common test for catalase is to add to the material under examination H_2O_2 and a substance like **benzidine** or **guaiac** which is easily oxidized to a colored compound. If catalase is present, the H_2O_2 is decomposed, and the resulting oxygen oxidizes the benzidine or guaiac. This oxidation is easily recognized by the color produced. A common application of this test is in determining whether milk has been heated. Normal milk contains catalase; but, if the milk has been heated to 80°C., the catalase is destroyed. Hence a catalase test on heated milk is negative.

Peroxidases. A simple system of oxidation has been suggested which involves peroxidases. These are found in many tissues; they decompose H_2O_2 and organic peroxides to form **atomic oxygen,** only in the presence of an oxidizable substance. In this respect they differ from catalase. The atomic oxygen produced oxidizes the substrate.

Since peroxidases decompose organic peroxides, it is evident that H_2O_2 is not necessary in testing for peroxidases. However, an organic peroxide must be present. In testing for peroxidase in a substance like potato extract benzidine is added, and the development of a blue color indicates a positive result. In the potato extract there is an auto-oxidizable substance which is oxidized by atmospheric oxygen to a peroxide. This, peroxidase enzyme decomposes to form atomic oxygen, which oxidizes the benzidine, giving the blue color. Chemically peroxidase is similar to catalase in that it is composed of a protein combined with heme.

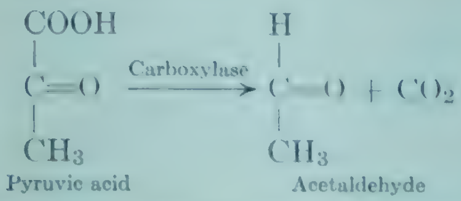
Tyrosinase. When freshly peeled potatoes are allowed to stand in the air, they turn black. This discoloration is due to the action of an oxidase enzyme called tryosinase, which catalyzes the oxidation of tyrosine by atmospheric oxygen. The reaction is a complicated one involving several steps, but the end result is a black oxidation product. Hydrogen peroxide is not formed in the reaction. Tyrosinase is of special interest because it contains copper. Chemically it is probably related to cytochrome and cytochrome oxidase, differing from them in that copper replaces iron in the molecule.

Desmolases

The desmolases, as mentioned above, are enzymes that break linkages not attacked by water. They break C—C, C—N, and other linkages. Included in this group are the **decarboxylases,** which break the C—C linkage, removing CO_2 from carboxyl groups, and the transaminases, which break the C—N linkage. Two decarboxylases are mentioned in the classification of enzymes, namely, **carboxylase** and

carbonic anhydrase. Although they are not oxidases, they are closely associated with biological oxidations.

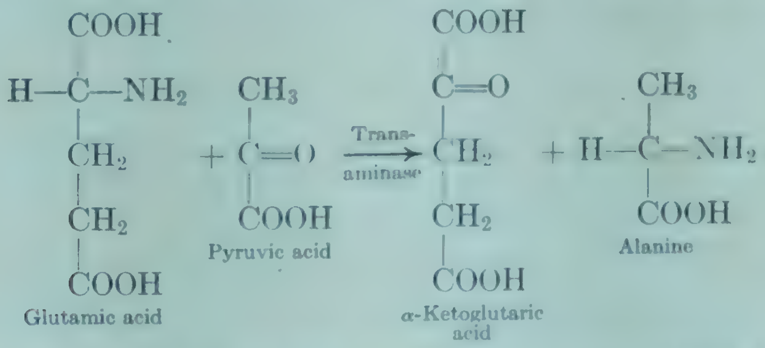
Carboxylase. Carboxylase occurs abundantly in plant and animal tissues, where it removes CO_2 from the carboxyl group of α-keto acids, leaving the corresponding aldehyde, thus:


COOH
C=O
CH3
Carboxylase
H
C=O
+ CO2
CH3
Pyruvic acid
Acetaldehyde

In order to work, carboxylase requires the presence of a divalent metal and a coenzyme called **cocarboxylase.** Cocarboxylase is a derivative of thiamine, or vitamin B_1, containing two molecules of H_3PO_4.

Carbonic anhydrase is found especially in red blood cells, where it controls the formation and decomposition of carbonic acid. It is thus a very important respiratory enzyme. Although it does not break a C—C linkage, it is so similar in its action to that of carboxylase that it is classed in the same group. Chemically it is a protein containing zinc.

Transaminases. The transaminases are important enzymes associated with protein metabolism. They catalyze the transfer of amino groups from amino acids, usually glutamic acid, to ketone acids and thus promote the formation of new amino acids. They require pyridoxal phosphate as a coenzyme. Pyridoxal phosphate is a derivative of pyridoxine, which is vitamin B_6. The following reaction is an example of a transamination catalyzed by **glutamic acid-alanine transaminase:**


COOH
H—C—NH2
CH2
CH2
COOH
Glutamic acid
CH3
+ C=O
COOH
Pyruvic acid
Trans-
aminase
COOH
C=O
CH2
CH2
COOH
α-Ketoglutaric acid
CH3
+ H—C—NH2
COOH
Alanine

It should be noted that in this reaction the C—N linkage is broken.

REVIEW QUESTIONS

1. Define catalyst and enzyme.
2. Discuss Buchner's work on yeast.

3. Distinguish between endo- and exoenzymes.
4. How are enzymes named?
5. What is the chemical nature of enzymes?
6. Define holoenzyme, apoenzyme, and coenzyme.
7. Distinguish between an activator and a kinase.
8. What are proenzymes?
9. What is an antienzyme?
10. Discuss the mechanism of enzyme action.
11. Discuss the specificity of enzymes.
12. Discuss the reversibility of enzyme action.
13. Name several factors influencing enzyme action.
14. Name the three main classes of enzymes and state the type of reaction with which each is associated.
15. Name several enzymes of each important class, telling where each is found, the substrate upon which it acts, and the end products formed.
16. What reaction does arginase catalyze?
17. What reaction does urease catalyze? Why is this reaction important to the biochemist?
18. What are cathepsins and why are they important?
19. What does rennin do? How is it used in the dairy industry and in the home?
20. What is bromelin, and for what reason is it important to the housewife?
21. How do amino- and carboxypolypeptidases differ in their activities?
22. Define oxidation. Give three examples of oxidation.
23. How do dehydrogenases differ from other oxidases with regard to the mechanisms of their action?
24. Show by means of an outline how dehydrogenase, coenzymes, flavoprotein, cytochrome, and cytochrome oxidase may function in a biological oxidation.
25. Indicate the chemical structure of coenzymes I and II. What is the active part of each molecule?
26. Indicate the chemical structure of a flavoprotein. What is the active part of the molecule?
27. What is the chemical nature of cytochrome and cytochrome oxidase?
28. What is glutathione? How may it function as an oxidation-reduction catalyst?
29. What is ascorbic acid? How may it function as an oxidation-reduction catalyst?
30. Name several vitamins associated with biological oxidations and reductions.
31. Why do peeled potatoes turn black when exposed to the air?
32. Distinguish between catalase and peroxidase.
33. What are desmolases? Name two types of desmolases.
34. With what reactions are carboxylase and carbonic anhydrase associated?
35. What is meant by transamination? Write an equation showing what happens during transamination.

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8

Digestion in the Mouth

General Considerations. Most of our foods are complicated chemical structures. In order that they may be absorbed and utilized by the body they must be broken down into relatively simple molecules. Carbohydrates must be hydrolyzed into monosaccharides, fats into glycerol and fatty acids, and proteins into amino acids. The chemical processes by which complex food materials are changed to simple molecules are called **digestion.**

In many of the lower forms of life, such as the bacteria, the absorption of digested foodstuffs takes place through the entire surface of the organism. In man and the higher animals absorption is limited to a very definite area, the inside of the digestive tract. The inside of the digestive tract is really part of the outside surface of the body. It is a highly specialized, delicate membrane through which digested food materials may diffuse with ease. Complex foodstuffs are hydrolyzed by means of enzymes, which are emptied into the digestive tract at various places along the route which the food must travel. These enzymes are many in number, and each plays its special role in the chemistry associated with the preparation of foods for utilization by the body.

Digestion during Food Preparation. Since digestion is the hydrolysis of complex food materials, it is evident that many of the processes involved in the preparation of food may be considered digestion. Cooking changes starch to dextrin and converts collagen to gelatin. The latter change takes place when tough meat is made tender by cooking. If the reaction of the mixture is alkaline, fats are hydrolyzed by cooking. An acidic medium greatly facilitates the hydrolysis of starch and disaccharides.

Cooking is an aid to digestion in another way. Starch is found in natural foods in the form of granules which are composed of soluble amylose and less-soluble amylopectin. Cooking ruptures the starch granules, exposing the amylose to the action of the digestive juices when the starch is eaten. Coagulation increases the digestibility of some proteins. For example, cooked eggs are more digestible than raw eggs.

Cooking destroys microorganisms which, if present in the food, might give rise to serious difficulties. Many cases of food poisoning have been due to the consumption of foods that had in them toxin-producing bacteria. This statement is especially true of meats and other high-protein foods.

During storage many important changes take place in foods. Meats stored under proper conditions become tender and much more desirable as food because of processes of **autolysis** which go on in the tissues after the death of the animal owing to the action of cathepsins. Many fruits and vegetables become much more palatable after storage as a result of enzymatic changes occurring during this period.

Saliva. The food enters the digestive tract through the mouth. Here it is chewed to break it up into small pieces and to mix it with the first of the digestive fluids, called the saliva. The saliva has two main functions: first, it is a slippery substance which is a great aid to swallowing; and, second, it contains an enzyme, salivary amylase, which hydrolyzes starch to maltose. (See Fig. 18, p. 212.)

The saliva is secreted by three pairs of salivary glands: the **parotid** under the ear, the **submaxillary** under the jaw, and the **sublingual** under the tongue. There are also numerous small **buccal glands** in the cheek. The saliva passes from these glands to the mouth by means of ducts. It has been estimated that a normal person secretes about 1500 ml. of saliva daily.

About 99.4 per cent of saliva is water. The protein material present is mainly **mucin,** a glycoprotein, which may be precipitated by the addition of dilute acid. Mucin is the constituent that makes saliva slippery. Saliva contains mineral salts. It is easy to demonstrate the presence of most of the inorganic salts of protoplasm in saliva by simple qualitative tests. The saliva of normal, healthy individuals is slightly acid in reaction, having a pH of about 6.5. It is believed by some persons that the acidity of saliva is a contributing factor in tooth decay; however, most authorities believe that the cause of dental caries is much more involved. Very likely, dental caries is due to a dietary deficiency.

The deposit of tartar on teeth is composed of calcium phosphate and calcium carbonate, together with small amounts of organic matter from the saliva. An alkaline saliva favors the formation of tartar.

Stimulation of the salivary glands may be brought about in various ways. The presence of food in the mouth stimulates the flow of saliva. Dry foods stimulate more than moist foods. The thought, sight, or smell of food will cause saliva to flow. Acids, salts, and many other chemical agents stimulate salivary secretion. To collect saliva for laboratory study, the salivary glands may be mechanically stimulated by chewing an inert substance, such as paraffin.

Many substances when taken into the body are excreted in the saliva. If a gelatin capsule containing potassium iodide is swallowed, in a short period of time the presence of iodides in the saliva may be demonstrated. The bitter taste after the injection of morphine is due to the excretion of the drug by the salivary glands. After drinking alcoholic beverages alcohol appears in the saliva in amounts proportional to those found in the blood. The analysis of saliva for its alcohol content has been used as a means of determining intoxication.

Salivary Amylase. The enzyme responsible for the digestive action of saliva is called salivary amylase (ptyalin). Actually salivary amylase appears to be a mixture of two enzymes, namely, α- and β-amylase, with α-amylase predominating. Salivary amylase acts on starch, breaking it down to **soluble starch, erythrodextrin, achroödextrin,** and finally **maltose.** It is important to note that salivary amylase does not completely hydrolyze starch to glucose but that maltose is the main end product formed. Maltose is later hydrolyzed by maltase in the intestine to glucose, in which form it is absorbed. Starch gives a blue color with iodine. As starch hydrolyzes the mixture gives a red color with iodine because of the formation of erythrodextrin. Finally there is no color reaction with iodine indicating that the starch has been broken down to achroödextrin which gives no color with iodine. Achroödextrin is finally broken down into maltose. It is believed that during hydrolysis the starch molecule is broken down by the removal of molecules of maltose. The reason for this belief is that maltose appears very early in the process.

There are several ways of studying the amylolytic activity of saliva. One is to determine the length of time required for starch to reach the **achromatic point,** or the point at which it no longer gives a color with iodine. Another is to follow the reaction by means of the polariscope, since the optical activity of a starch solution changes as hydrolysis proceeds. Still another method is to study the change in viscosity of the starch solution. Starch solution has a high viscosity; as it hydrolyzes, the viscosity decreases. A very good quantitative method is to study the reducing power of the solution, as digestion proceeds, with Fehling's solution. Starch does not reduce Fehling's solution, but maltose does. Maximum reduction indicates that the reaction has been completed.

Salivary amylase acts rapidly at body temperature, but at 50 to 55°C. it acts most rapidly. At 75°C. its activity is destroyed. It is active over a pH range of 4 to 9, the optimum being 6.6. Since the acidity in the stomach reaches a pH of less than 2.0, amylase activity is soon inhibited in the stomach. In fact, the acidity in the normal stomach is sufficient to permanently destroy amylase activity.

The chloride ion is essential for amylase activity. If it is removed by dialysis, amylase activity ceases. Activity returns upon the addition of the chloride ion.

Certain carnivorous and herbivorous animals such as dogs and sheep have no amylase in their saliva. Enzymes other than amylase have been demonstrated in the saliva, but they are of little importance. Catalase is present, as evidenced by the liberation of oxygen from hydrogen peroxide when it is used as a gargle. Traces of maltase, lipase, and protease have been demonstrated to be present in saliva.

The importance of salivary digestion and the thorough chewing of food has been underestimated. When the rapidity with which salivary amylase hydrolyzes starch *in vitro* is considered, it cannot be doubted that considerable digestion of starch goes on in the mouth even with normal chewing. Since salivary amylase acts until the *p*H reaches about 4.0, it is evident that considerable action may take place in the stomach before acidity develops sufficiently to inhibit it. This is especially true of lumps of food which have been well mixed with saliva. It has been estimated that amylase activity may continue for 30 minutes after food is swallowed. Any starch which has not been hydrolyzed in the mouth or stomach by salivary amylase is digested in the intestine by pancreatic amylase.

Since in passing from the stomach to the intestine the reaction of the digesting mixture of food changes from a *p*H of about 2.0 to about 7.0, it would appear that salivary amylase is reactivated in the intestine. However, this is not the situation. The acidity of the normal stomach is sufficient to destroy amylase activity completely.

There is evidence that pepsin activity is greatly aided when the food is well mixed with saliva. Why this is so is not known. It therefore appears that the time devoted to masticating food thoroughly is well spent.

REVIEW QUESTIONS

1. Define digestion.
2. Discuss digestion during food preparation.
3. Name two important constituents of the saliva and state the function of each.
4. Name several methods that may be used to determine amylase activity.
5. What is the *p*H of normal saliva?
6. What are the optimum conditions for the action of salivary amylase?
7. Does salivary amylase continue to act in the stomach? In the intestine?
8. Name the substrate for and the main end product of salivary amylase activity.

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9

Digestion in the Stomach

Structure of the Stomach. Upon leaving the mouth the food passes through the esophagus into the stomach. Structurally the stomach is made up of two parts. (See Fig. 18.) The part to which the esophagus is attached is called the **fundus.** The part leading into the intestine is called the **pylorus.** The fundus constitutes the main body of the stomach; the pylorus is a more or less constricted area leading into the intestine. The walls of the stomach are muscular, and when empty the organ is collapsed. As food enters, the walls of the stomach distend, allowing considerable capacity for the storage of food. One of the main functions of the stomach is as a storage organ for undigested food. The food remains in the stomach from 1 to 5 hours, being passed on to the intestine slowly. When water alone is taken, it passes through the stomach rapidly. Solid food collects in the fundus portion of the stomach.

Beaumont's Work on Gastric Digestion. The first important work on gastric digestion was done by Dr. William Beaumont, an American army surgeon, on Mackinac Island at the head of Lake Michigan, in 1822. As a result of the accidental discharge of a musket, Alexis St. Martin was wounded in the left side in such a manner that upon recovery an opening was left through the abdominal wall into his stomach. Realizing the opportunity to study gastric digestion, Dr. Beaumont hired St. Martin to submit to many experiments with the result that much information was gained regarding the physiology of the stomach. It is interesting to note that most of Beaumont's findings have been confirmed by later workers.

Beaumont found that the lining of the empty stomach was covered with a layer of mucus which was neutral or alkaline in reaction. When food entered the stomach or when the stomach lining was stimulated by mechanical irritation the membrane became bright red and a colorless juice welled up from the openings of small glands. This juice was distinctly acidic in character, and the acid present proved to be hydrochloric acid. He collected samples of the juice and found that they dissolved meat in a test tube. When applied to wounds the juice kept them clean, hastened healing, and prevented pus formation.

He studied the time required to digest meals of various types and found that fatty foods required a longer time to digest than lean. After a meal of mutton chops and potatoes, the stomach was empty in 2 hours, whereas after a meal of roast pork, potatoes, vegetables, and pudding, 4 or 5 hours elapsed before the stomach was empty. After the consumption of large quantities of alcoholic liquor, the stomach lining became inflamed, small ulcers appeared, and the amount of gastric juice secreted was small and of low digestive ability. During fever and constipation gastric digestion was greatly retarded.

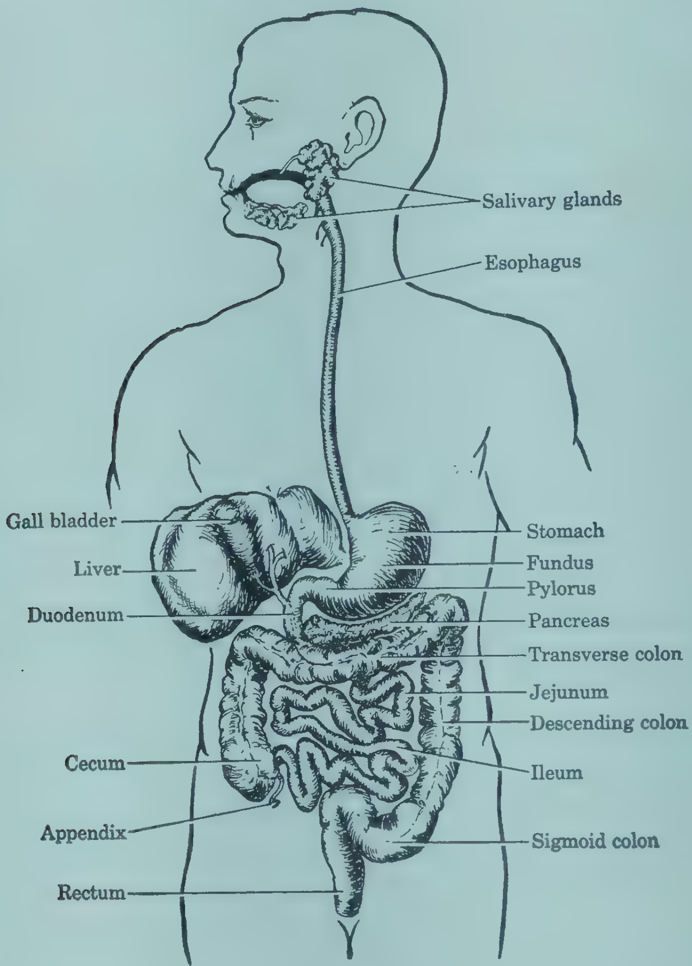
Salivary glands
Esophagus
Gall bladder
Liver
Duodenum
Stomach
Fundus
Pylorus
Pancreas
Transverse colon
Jejunum
Descending colon
Cecum
Ileum
Appendix
Sigmoid colon
Rectum

FIG. 18. The digestive tract.

Beaumont observed no appetite secretion of gastric juice. In other words, the thought, odor, or taste of food did not cause gastric juice to flow. He believed that the sensation of hunger was produced by the turgidity of the gastric glands.

Gastric Secretion. Although the stomach functions as a storage organ, very important digestive changes take place in it. Scattered throughout the walls of the stomach are numerous small glands which secrete a digestive fluid known as the **gastric juice.** There are three types of cells found in gastric glands, namely, (1) **parietal** cells which secrete hydrochloric acid, (2) **chief** cells which produce pepsin and possibly other enzymes, and (3) **mucous** cells which secrete mucin. There is a difference of opinion as to whether gastric juice is secreted when there is no food in the stomach. The fact that the resting stomach usually contains about 50 ml. of a liquid often spoken of as the residuum has been taken to mean that there is a constant flow of small amounts of gastric juice. Various stimuli will affect the rate of flow of gastric juice. First, contrary to Beaumont's finding, there is a psychic stimulation; the thought, sight, and taste of food will increase the flow of gastric juice. Second, the presence of food in the stomach has an effect on gastric secretion, the amount of secretion varying with the kind of food. Third, the presence of protein digestion products in the intestine stimulates gastric secretion.

There is evidence indicating that the presence of food in the pylorus and intestine causes the production in the mucous membrane of a substance called **gastrin,** which enters the blood and acts as an internal messenger, or **hormone,** stimulating the gastric glands. This stimulation of the flow of gastric juice by gastrin continues for some time and is perhaps one of the most important factors causing gastric secretion. Gastrin is thought by some to be **histamine,** which, when injected into the blood, stimulates gastric secretion.

The presence of undigested fat in the intestine inhibits gastric secretion. This effect is due to a hormone, called **enterogastrone,** liberated from the intestinal mucosa. The amount of gastric juice secreted per day varies greatly, depending on the diet. Usually 2 to 3 liters is secreted every 24 hours.

General Nature of Gastric Juice. Normal gastric juice is a light-colored, thin fluid decidedly acidic in reaction. It contains the enzymes **pepsin** which acts on proteins, **rennin,** in some species which acts on casein causing milk to curdle, and a **lipase** which hydrolyzes fat. The most important is pepsin.

Gastric Acidity. The acidity of pure gastric juice is due to hydrochloric acid. When gastric juice is mixed with foods, other acids may be present, such as lactic acid, produced by fermentation. Hydrochloric acid is produced by the parietal cells of gastric glands whose secretion is an almost pure solution of HCl, having a concentration of about 0.17 *N* and a *p*H of 0.87. When mixed with the secretion of the chief and mucous cells and with food, the acidity drops so that the *p*H of normal stomach contents is about 1.6 to 1.8. If the acidity is greater, **hyperacidity** of the stomach is said to exist. This condition is common in ulcers of the stomach. If the acidity is less than normal, the condition is known as **hypoacidity.** This is common in cancer of the stomach and pernicious anemia.

Frequently, in medicine the acidity of the gastric contents is determined as a diagnostic procedure. On an empty stomach the patient is given a test meal consisting of toast and tea. An hour later a stomach tube is swallowed, and the stomach contents are removed. The material is filtered through cheesecloth, and portions of the solution are titrated with 0.1 *N* alkali. By using indicators which change color at different *p*H values, different types of acidity may be determined. The most common procedure is to determine total acidity with **phenolphthalein** as the indicator, and free HCl with **Töpfer's reagent** as the indicator. Phenolphthalein gives the total acidity because this indicator changes color on the alkaline side of neutrality, at a *p*H of about 9.0; and Töpfer's reagent indicates free HCl because it changes color at a *p*H of about 3.0 to 4.0. With this procedure gastric acidities are expressed as milliliters of 0.1 *N* alkali required to neutralize 100 ml. of juice. Normal gastric contents have a total acidity of about 60 and a free HCl acidity of about 50.

Origin of Gastric Acidity. The blood plasma and protoplasm of the tissues are slightly alkaline in reaction, having a *p*H of 7.35. The secretion of the parietal cells is highly acid in character, having a *p*H of 0.87. The question of how such an acidic solution can be derived from an alkaline medium is an interesting one. Many theories have been advanced to explain the origin of HCl in the gastric juice, but only one will be discussed here. According to Hollander, HCl is produced in the parietal cells by the hydrolysis of chlorides such as NaCl.

$$\mathrm{NaCl + H_2O \rightarrow HCl + NaOH}$$

The NaOH formed is immediately neutralized by reacting with buffers such as NaH_2PO_4 and $NaHCO_3$.

$$\mathrm{NaOH + NaH_2PO_4 \rightarrow Na_2HPO_4 + H_2O}$$

According to Hollander, the secreting membrane of the parietal cell is permeable to H_2O, H^+, and Cl^- in one direction only. When a parietal cell is stimulated to secrete, water is forced out of the cell in two directions. That which goes forward into the secretion carries H^+ and Cl^-

with it, while that which goes backward into the tissues carries alkalinized buffers. Thus, as acid is being removed from the parietal cell, basic factors are also being removed, and the *p*H of the cell remains constant.

The absorption of alkalinized buffers into the blood and tissues, following the secretion of gastric juice, tends to increase their *p*H. This temporary increase in alkalinity is often spoken of as the **alkaline tide.** The feeling of well-being after a hearty meal is said to be due to this alkaline tide.

Pepsin. The most important enzyme in the gastric juice is pepsin. Pepsin hydrolyzes proteins into proteoses and peptones, mainly. Pepsin attacks peptide linkages in which **tyrosine** and **phenylalanine** are involved. If these amino acids are at the end of a protein molecule, the free amino acids are liberated; if they are located in the central portion of the molecule, the split occurs there, leaving shorter polypeptide chains. Thus the size of the molecules resulting from the action of pepsin on proteins depends on the location of tyrosine or phenylalanine in the substrate.

Pepsin is secreted in an inactive form called **pepsinogen.** The HCl in the gastric juice converts pepsinogen to the active enzyme pepsin. That it is the hydrogen ion that activates pepsinogen is shown by the fact that other acids than HCl have a similar effect. After the hydrogen ion has converted some pepsinogen into pepsin, the pepsin becomes active in converting more pepsinogen into pepsin. This is spoken of as an **autocatalytic reaction.** Like other enzymes, pepsin requires a rather definite *p*H for its greatest activity. The optimum lies between 1.5 and 2.0. At a *p*H of 4.0 peptic activity nearly ceases. Since the *p*H of normal gastric contents varies from 1.6 to 1.8, it is seen that the normal stomach is ideal for the action of pepsin. Both pepsinogen and pepsin have been prepared in crystalline form. They are both proteins.

It is often desirable to determine **peptic activity** in a gastric analysis. This is usually done by **Mett's method,** in which fine glass tubes containing coagulated egg albumin are placed in a beaker, together with a sample of the gastric contents. This material is incubated at 37° C. for 24 hours, and at the end of that time the length of the column of albumin digested is determined by means of a millimeter scale. Results are expressed as the square of the millimeters of albumin digested.

Other methods for studying peptic activity involve the **Sörensen titration** and the **Van Slyke amino nitrogen** determination. The values obtained in both these determinations increase as protein hydrolysis progresses and finally reach a maximum when hydrolysis is complete. Another method involves the determination of the nonprecipitable nitrogen produced when pepsin acts upon a precipitable protein substrate, such as egg albumin solution.

Rennin. Rennin is another proteolytic enzyme found in the gastric juice of some animals. It is found in the fourth stomach of calves and possibly of other ruminants. The action of rennin is to clot milk. Commercial rennin, used in cheese making and for preparing junket in the home, is prepared from calves' stomachs. Formerly it was thought that rennin occurred in the human stomach, but it is now believed that the clotting of milk which occurs in the human stomach is caused by pepsin or by the acid present in the gastric juice. Pepsin will clot milk at a *p*H of 5, in a manner similar to that of rennin.

When milk reaches a *p*H of 4.6 to 4.8, casein is at its isoelectric point and precipitates. Under these conditions, casein is precipitated as such and not as calcium paracaseinate. The acidity of the stomach contents is more than sufficient to cause this acid coagulation of casein. The curdling of sour milk is due to the acidity produced by lactic acid formed in the bacterial fermentation of the lactose in milk.

Rennin acts on a protein of milk, **casein,** changing it into **paracasein.** Paracasein combines with calcium to form **calcium paracaseinate,** which is insoluble. With the precipitation of calcium paracaseinate the milk is said to curdle. If calcium salts are precipitated from milk by the addition of oxalate, rennin will not curdle milk. However, if calcium salts are added after rennin has acted on oxalated milk, a clot is immediately formed. This reaction shows that calcium is not necessary for rennin to act on casein but is necessary for the precipitation of the paracasein. Rennin acts best at a *p*H of 6.0 to 6.5. The curdling of milk in the stomach is important in digestion because in curdled form the milk remains in the stomach for a longer period of time than uncurdled milk would. This gives the gastric enzymes a longer time to act on milk.

Gastric Lipase. The gastric secretion contains a fat-hydrolyzing enzyme known as gastric lipase. The amount of fat hydrolyzed in the stomach is small, and only fats which are in an emulsified form are attacked. A good example of such a fat is that found in milk. Fats do not emulsify well in an acidic medium; hence emulsification does not take place to any great extent in the stomach. If for any reason the gastric acidity is low, lipase activity is increased. Gastric lipase is most active at *p*H 5.

Other Digestion in the Stomach. Food entering the stomach is mixed with saliva, which contains salivary amylase. This continues to act on starch for some time, converting it into maltose, especially inside the masses of food which are swallowed. As time goes on, the acidity of the stomach reaches a point where salivary amylase activity is inhibited, but during this period of increasing acidity considerable starch digestion takes place.

Sucrose is easily hydrolyzed by acid, and undoubtedly some digestion of this sugar takes place in the stomach with the formation of glucose and fructose.

Under certain conditions the intestinal juice regurgitates into the stomach. If this occurs, some intestinal digestion may take place in the stomach. A question frequently asked is: What prevents the stomach from digesting itself, since the stomach walls are made of protein, which should be digested by a proteolytic enzyme? There are two explanations. First, the protoplasm of the living cells is alkaline in reaction. Since pepsin requires an acid medium, conditions are not correct in the living cells for its action. Second, the living cells contain an antienzyme which inhibits the action of pepsin.

Germicidal Action of the Gastric Juice. Many of the foods which we eat contain microorganisms. In a stomach with normal acidity most of these microorganisms are killed. Most of the disease-producing bacteria are rather delicate and undoubtedly are destroyed in the normal stomach. The acidity in the stomach is an important provision of nature to prevent intestinal infections. If the acidity of the stomach contents is low, fermentations occur with the production of gas, which may give great discomfort. A common product of fermentation in the stomach is **lactic acid**; hence a test for this acid in a gastric analysis is of importance.

Gastric Absorption. Very little absorption of food materials takes place in the stomach. Some monosaccharides, salts, and drugs may be absorbed. As will be discussed later, most foods are absorbed from the small intestine.

Evacuation of the Stomach. As gastric digestion proceeds, the food becomes more or less liquefied. The liquefied stomach contents are called **chyme.** The stomach wall begins to contract, producing a peristaltic wave which moves toward the pyloric opening into the intestine. As digestion proceeds, these peristaltic waves become more powerful, and the more liquid chyme is forced into the intestine. The movement of the stomach mixes the contents and in this way is a very valuable aid to digestion. Finally all the food is liquefied, and all the chyme is forced into the intestine. The time required for gastric digestion and evacuation of the stomach varies with the type of food consumed. An easily digested meal consisting mainly of carbohydrates requires about 2 hours, whereas a heavy meal of fatty foods and meat requires 4 or 5 hours for passage through the stomach.

REVIEW QUESTIONS

1. Describe the structure of the stomach.
2. Describe Beaumont's work on gastric digestion.
3. Name three types of cells found in gastric glands and tell what each type secretes.
4. Discuss gastric secretion. What factors influence it? What are gastrin and enterogastrone?
5. Name the enzymes found in the gastric juice.
6. What is the *p*H of normal gastric contents?
7. What is meant by hypo- and hyperacidity?
8. How are total acidity and free HCl determined in a gastric analysis?
9. Give Hollander's theory explaining the origin of HCl in the gastric juice.
10. What is pepsinogen? Give two methods the body has of converting this substance into pepsin.
11. What does pepsin do, and how may peptic activity be measured?
12. Is rennin present in human gastric juice? How is casein precipitated in the human stomach?
13. For what is rennin used in the dairy industry and in the home?
14. Explain the chemistry involved in the precipitation of casein by rennin.
15. What is the action of gastric lipase?
16. What digestion may take place in the stomach other than that produced by the enzymes of the gastric juice?
17. State the importance of the germicidal action of the gastric juice.
18. Discuss gastric absorption.
19. What is chyme?
20. How long does food normally stay in the stomach?
21. How does the stomach empty itself?
22. Since the stomach is made of protein and contains proteolytic enzymes, how do you account for the fact that it does not digest itself?

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Digestion in the Intestine

The chyme, as it leaves the stomach, passes into the small intestine. For digestion, the most important part of the small intestine is the first 11 or 12 inches, called the **duodenum.** This name comes from a Latin word meaning twelve, which suggests its length. The next section of the small intestine is called the **jejunum;** the main part is called the **ileum.** The ileum empties into the **cecum,** which is the first part of the large intestine, or **colon.** The colon empties into the **rectum.** (See Fig. 18, p. 212.)

Three juices enter the intestine in the duodenum. They are the **intestinal juice,** secreted by the duodenal wall itself; the **pancreatic juice,** the external secretion of the pancreatic gland; and the **bile,** which comes from the liver. All these juices play an important part in digestion. All are alkaline in reaction and tend to neutralize the acidity of the chyme.

The Intestinal Juice. The intestinal juice is secreted mainly in the duodenum, although some is secreted in the jejunum and ileum. There are two types of intestinal secretion. One, which is independent of food intake, occurs every 2 hours. This secretion is low in digestive power but is perhaps important for the normal functioning of the bowels. The other secretion is concerned with digestion and is secreted whenever food enters the intestine. This juice has only mild digestive activity.

There are three ways in which the flow of intestinal juice may be stimulated. First, the presence of food in the intestine causes a mechanical stimulation of the mucosal glands; second, a hormone called **enterocrinin,** secreted by the intestinal mucosa, stimulates the mucosal glands; and third, a hormone called **secretin,** which stimulates the pancreas to produce pancreatic juice and the liver to produce bile, also stimulates the mucosal glands to produce intestinal juice.

The enzymes present in the intestinal juice are the **peptidases,** which hydrolyze various peptides to amino acids; three disaccharide-splitting enzymes, **sucrase, maltase,** and **lactase;** and **nucleases,** which hydrolyze nucleic acid. **Enterokinase,** an enzyme which converts the inactive proteolytic proenzyme of the pancreatic juice, **trypsinogen,** into active **trypsin,** is also present in the intestinal juice.

Peptidases. Two peptidases which are associated with the hydrolysis of polypeptides to amino acids are found in the intestinal juice. One, called **aminopolypeptidase,** hydrolyzes polypeptides to amino acids and simpler peptides by removing the amino acid containing the free amino group from the polypeptide. When the polypeptide has reached the dipeptide stage, as a result of repeated action of aminopolypeptidase, the dipeptide is finally hydrolyzed by the other proteolytic enzyme in the intestinal juice, which is called **dipeptidase.** The intestinal peptidases act best at a *p*H of 7.0 to 8.0. It should be noted that the peptidases do not act on native proteins but only complete the hydrolysis started by other proteolytic enzymes.

Sucrase, Maltase, and Lactase. The common disaccharides found in food, which must be hydrolyzed before they are available to the body, are sucrose, maltose, and lactose. Sucrose is consumed as cane sugar; maltose is derived from the partial hydrolysis of starch by the amylases of the saliva and of the pancreatic juice; and lactose is found in milk. The three enzymes necessary for the hydrolysis of these disaccharides are present in the intestinal juice. Of these three, sucrase and maltase are present in large quantities; lactase, in small quantities. Lactase can most easily be demonstrated in the intestinal juice of young animals, for which milk is the main article of the diet. As the animal becomes older, the lactase activity becomes more feeble. Lactase appears to be more intimately associated with the intestinal mucosa than sucrase and maltase are. Sucrase acts best at a *p*H of 5.0 to 7.0, maltase at 6.7 to 7.2, and lactase at 5.4 to 6.0.

Nucleases. Three enzymes responsible for the hydrolysis of nucleic acid are present in the intestinal juice. They are: (1) **polynucleotidase,** which hydrolyzes nucleic acid into nucleotides; (2) **phosphatase,** which removes phosphoric acid from nucleotides, forming nucleosides (it also hydrolyzes other organic phosphates such as hexose and glycerophosphates); and (3) **nucleosidase,** which hydrolyzes purine nucleosides to sugar and purine. No enzyme capable of hydrolyzing pyrimidine nucleosides is present in the intestinal juice.

Enterokinase. A very important constituent of the intestinal juice is enterokinase, an enzyme that converts trypsinogen of the pancreatic juice into the active proteolytic enzyme trypsin.

Importance of the Duodenum. The duodenum is undoubtedly a very important organ. If it is removed, the animal dies in a few days.

For some unknown reason the duodenal secretion is necessary for life. In nephritis, where the kidney function is impaired, the duodenum may play an important part in removing those waste products of metabolism which are ordinarily excreted through the kidneys. For this reason it is very important that the bowels be kept open in patients suffering from kidney disease. Many substances, such as certain drugs and metals, when injected into the blood find their way out of the body by the route of the duodenal secretion.

The Pancreatic Juice. The pancreas is a glandular organ lying close to the duodenum. It produces two secretions: one, an internal secretion called **insulin,** passes directly into the blood stream and is essential for the proper utilization of carbohydrates; the other, an external secretion called the **pancreatic juice,** is carried by a duct into the duodenum and is important in the digestion of foods. The pancreatic juice is an alkaline liquid having a pH of 7.5 to 8.0.

The flow of pancreatic juice is not continuous but occurs only when acidic chyme enters the duodenum. In the duodenal mucosa there is a hormone called **secretin** which is liberated by the HCl of the chyme. Secretin is carried by the blood to the pancreas, which it stimulates to produce pancreatic juice. That such a hormone is actually present in the blood stream has been proved by crossing the circulation of two dogs. If the duodenum of one dog is stimulated by acid, pancreatic juice is produced in both dogs. Then again a neutralized acid extract of the duodenum, when injected into the blood stream of a dog, will cause a production of pancreatic juice.

Another hormone obtained from intestinal tissue, called **pancreozymin,** controls the enzyme-producing function of the pancreas. Whereas secretin controls the volume of pancreatic juice secreted, pancreozymin controls the amount of enzymes which it contains.

The amount of pancreatic juice produced per day is variable and is apparently related to the kind of food eaten. Since gastric secretion likewise varies with the type of food eaten, it is likely that gastric and pancreatic secretions are closely related. When more gastric juice is produced, acidic chyme enters the duodenum for a longer period of time, and thus the stimulation of the pancreas is prolonged. Under normal conditions the amount of pancreatic juice produced per day varies from 500 to 800 ml.

The pancreatic juice contains enzymes for the digestion of **proteins, fats, carbohydrates,** and **nucleic acid.**

Pancreatic Proteases. There are three proteases in the pancreatic juice, namely, **trypsin, chymotrypsin,** and **carboxypolypeptidase.** Trypsin and chymotrypsin are secreted in inactive forms or as proenzymes called **trypsinogen** and **chymotrypsinogen.** These three enzymes and two proenzymes have been obtained in crystalline form; they are protein in nature. Trypsinogen is converted into trypsin by the enzyme enterokinase of the intestinal juice. Chymotrypsinogen is activated by trypsin. Active trypsin also has the power of activating trypsinogen in a manner similar to the activation of pepsinogen by pepsin in the stomach. Both trypsin and chymotrypsin attack **native proteins**, hydrolyzing them to **proteoses, peptones, polypeptides,** and some amino acids. They attack specific peptide linkages in the protein molecule. Trypsin hydrolyzes peptide linkages involving the carboxyl groups of **arginine** and **lysine;** chymotrypsin, those involving the carboxyl groups of **tyrosine** and **phenylalanine.** Both enzymes curdle milk, but chymotrypsin is much the more powerful in this respect. Trypsin acts best at a pH of 8.0 to 9.0.

Carboxypolypeptidase hydrolyzes polypeptides to simpler peptides and amino acids by the successive removal of the amino acid containing the free carboxyl group at the end of the polypeptide chain.

Pancreatic Lipase. The pancreatic juice contains a lipase formerly called steapsin, which hydrolyzes fats into **glycerol** and **fatty acids.** Its activity is greatly increased by the presence of bile salts. Pancreatic lipase is by far the most important enzyme concerned with fat digestion. Very little fat digestion is due to gastric lipase.

According to Frazer, fats are not completely hydrolyzed by pancreatic lipase; they are only partially broken down into mono- and diglycerides and fatty acids. Frazer believes that undigested fat molecules, including mono- and diglycerides, may be absorbed in an emulsified form.

Pancreatic Amylase. The pancreatic juice contains a starch-splitting enzyme, similar to salivary amylase. It was formerly called amylopsin but now is usually referred to as pancreatic amylase. It hydrolyzes **starch** to **maltose.** It works best in a neutral to slightly alkaline medium. The disaccharide-splitting enzymes, maltase, sucrase, and lactase, have been found in pancreatic juice, but their main source is the intestinal juice.

Pancreatic Nucleases. Two polynucleotidases are present in pancreatic juice. One hydrolyzes ribonucleic acid and the other desoxyribonucleic acid into their constituent nucleotides.

The Bile. We have just studied the intestinal and pancreatic juices, which play an important role in the digestion of food. In addition to these juices a third fluid is poured into the duodenum which is of importance in digestion, although alone it has no digestive action. This fluid is the bile, which comes from the liver. The bile passes from the liver to the intestine through a duct. Alongside this duct, and opening into it, is the gall bladder which acts as a reservoir for storing bile at times when it is not needed in the intestine. Bile is produced continuously by the liver, but the amount varies with the diet. In general it may be stated that protein foods cause a greater production of bile than carbohydrate foods. From 500 to 1000 ml. of bile is produced per day by a normal adult. When food enters the duodenum, the liver is stimulated to produce more bile, and the gall bladder is caused to empty by a contraction of its walls. The stimulation of the liver to produce bile is said to be caused by the hormone, **secretin**, which has already been discussed in connection with the stimulation of the pancreas. The emptying of the gall bladder is controlled by a hormone called **cholecystokinin,** produced in the duodenal mucosa. The gall bladder is not merely a reservoir for bile. Substances in the bile which are of value to the body are reabsorbed here, and there is an absorption of water resulting in a concentration of the bile. The gall bladder also excretes certain substances into the bile, thereby acting as an excretory organ.

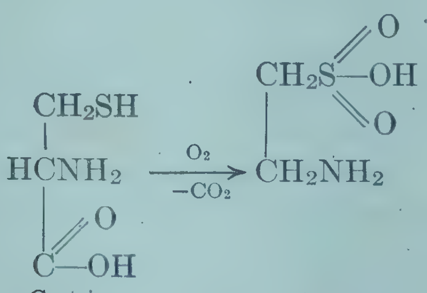
The bile is a viscid liquid, alkaline in reaction and bitter in taste. Its color varies from yellowish brown to green. Its important constituents are the **bile pigments,** the **bile salts,** and **cholesterol.** Certain metallic substances and toxins may also be present. The bile may be looked upon both as a secretion and an excretion: the bile salts which are important in the digestion and absorption of fats should be considered a secretion; the bile pigments and cholesterol, which apparently have no function in the intestine and are largely eliminated from the body in the bile, should be considered excretions.

The Bile Pigments. The main pigment in the body is the **hemoglobin** of the blood. This is a compound protein composed of the protein **globin** and the red coloring matter **heme.** Hemoglobin is contained within the red blood cells. The average life of a red blood cell is about 4 months. As red blood cells decompose, hemoglobin is liberated and is converted into the bile pigments, **biliverdin** and **bilirubin.** These pigments are derived from the heme portion of hemoglobin. Heme contains iron of which the body is very economical. The bile pigments contain no iron. Thus before heme is discarded by the body, its iron is removed to be used again in the synthesis of hemoglobin. The original bile pigment is biliverdin, which is green. In some animals biliverdin is the main bile pigment, and the bile is green. In other animals, including man, much of the biliverdin is reduced to bilirubin, which is yellow, and the bile appears yellow. When the bile reaches the intestine, further changes take place owing to bacterial action. Bilirubin is reduced to **mesobilirubinogen,** which on further reduction gives **stercobilinogen.** This on oxidation gives **stercobilin,** the brown pigment responsible for the color of feces. Some of the stercobilinogen and stercobilin is reabsorbed into the blood from the intestine and is re-excreted by the liver. Some is excreted in the urine, accounting for its color. In the urine these pigments are called **urobilinogen** and **urobilin.** The occasional production of green feces in infants with diarrhea is due to biliverdin and indicates that oxidation, instead of the normal reduction, has taken place in the intestine.

The conversion of hemoglobin into bilirubin occurs in the liver and other tissues. The black-and-blue spot caused by an injury demonstrates the conversion of hemoglobin into the bile pigments. When tissue is injured, blood escapes from the capillaries and decomposes. First a bluish red color is noticed, which finally changes green, yellow, and brown. Thus several of the bile pigments mentioned may be observed in an ordinary black eye.

If there is an obstruction to the flow of bile, if liver function is impaired, or if red blood cells are being destroyed more rapidly than is normal, bile pigments will accumulate in the blood, and the skin will become yellow. This condition is known as **jaundice.** The severity of jaundice may be determined by comparing the color of the blood serum with a 0.01 per cent $K_2Cr_2O_7$ solution. The number of times the serum must be diluted with physiological salt solution in order to match the color of the $K_2Cr_2O_7$ standard is called the **icteric index.** Thus, if 1 ml. of serum must be diluted to 10 ml. to match the standard, the icteric index is 10. The icteric index of a normal individual is between 4 and 6.

The Bile Salts. The main bile salts are the sodium salts of **glycocholic** and **taurocholic acids.** These acids are complicated structures composed of **glycocoll** and **taurine** in combination with **cholic acid.** Glycocoll is another name for the simplest amino acid, glycine. Taurine is the amino acid cysteine with the carboxyl group removed and with the sulfur oxidized from a valance of 2 to 6. It is derived from cysteine, thus:



Cysteine Taurine

In the bile acids glycocoll and taurine are linked to cholic acid by a peptide linkage. There are many other bile salts besides those we have mentioned. These others are different in that, instead of cholic acid, they contain modified cholic acids. Cholic acid is a sterol; its formula has been given. (See p. 131.)

The bile salts are the most important constituents of the bile from the standpoint of digestion. In the presence of bile salts the activity of pancreatic lipase is greatly increased. This increase in activity appears to be due to the emulsification of fats in the presence of the bile salts. The bile salts lower the surface tension, thus aiding emulsification.

Another important function of the bile salts is to aid in the absorption of fatty acids. Fatty acids in combination with bile salts are soluble and can be absorbed through the intestinal wall in this form. The bile salts, which are thus reabsorbed, are carried to the liver, where they are again secreted in the bile. Thus there is a circulation of the bile salts in the body.

By aiding fat digestion the bile salts also aid indirectly the digestion of other food constituents. If fat is present in food in quantity, it may coat other food particles, thus preventing the action of other enzymes.

Cholesterol. The third important constitution of the bile is cholesterol. Little is known about its origin in the animal body. That the body can synthesize it is indicated by the fact that more cholesterol may be found in bile than is present in the food. Of special interest is the relation of cholesterol to **gallstones.** Although there are many kinds of gallstones, the most common type found in humans are composed largely of cholesterol. Cholesterol in the bile is considered a waste product, which is removed from the body through the liver. A reduced cholesterol called **coprosterol,** which is derived from cholesterol in the intestine, is found in the feces.

Several theories have been advanced concerning the origin of gallstones. One is that they are a result of infection in the gall bladder. When infection occurs nuclei are produced upon which cholesterol may deposit, forming stones. Another theory is that they are a result of faulty metabolism. Cholesterol is held in solution in the bile by means of the bile salts. If too much cholesterol is excreted in the bile or if the concentration of the bile salts is low, cholesterol may precipitate. A third theory is that they are a result of stagnation of the bile in the gall bladder. When this occurs bile salts may be reabsorbed into the blood stream, leaving the cholesterol without sufficient bile salts to hold it in solution.

REVIEW QUESTIONS

1. Name the regions into which the intestines are divided.
2. Name the juices, important to digestion, which enter the intestine.
3. Name three ways in which the flow of intestinal juice may be stimulated.
4. Name the enzymes present in the intestinal juice and state the function of each.
5. What is enterokinase and what is its function?
6. With what reaction is aminopolypeptidase associated?
7. Name the enzymes involved in the hydrolysis of nucleic acid and state what each does.
8. With what reaction is phosphatase associated?
9. Name the internal and external secretions of the pancreas.
10. Discuss the importance of the duodenum as an excretory organ.
11. How is the flow of pancreatic juice controlled?
12. Name all the enzymes found in the pancreatic juice and state the function of each.
13. How are trypsinogen and chymotrypsinogen converted into trypsin and chymotrypsin, respectively?
14. What peptide linkages in the protein molecule do trypsin and chymotrypsin attack?
15. According to Frazer, what is the action of pancreatic lipase on fats?
16. How is the liver stimulated to produce bile?
17. What is the function of cholecystokinin?
18. Name the three main constituents of the bile.
19. Name the bile pigments. How are they related to each other chemically, and from what do they originate?
20. What do the bile pigments change to in the intestine?
21. What are the pigments in the urine and what is their origin?
22. What are jaundice and the icteric index?
23. Name the bile salts and indicate their chemical constitution.
24. Of what importance are the bile salts in digestion?
25. What is meant by the circulation of the bile salts?
26. What are gallstones? Give three theories as to their origin.

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11

Absorption

The inside of the digestive tract is in reality a specialized area of the exterior surface of the body. When we have swallowed food, it has not entered the living tissues of the body. The passage of food through the lining of the digestive tract into the blood and tissues is called **absorption.**

No absorption takes place in the mouth, and very little occurs in the stomach. There is possibly slight absorption of simple molecules, such as glucose, through the stomach wall, provided that such substances are present in the stomach contents.

Practically all absorption takes place in the small intestine. Little food remains to be absorbed by the time the contents of the small intestine reach the large intestine. The small intestine offers a large surface area for absorption. Its apparent area is increased from three to twelve times by fingerlike projections called **villi** (see Fig. 19), which project into the lumen of the intestine. It is through these villi that most absorption takes place. In the center of each villus is a vessel called a **lacteal,** which is filled with a colorless fluid, **lymph.** Around the lacteal is a network of capillaries, by means of which the blood is brought very close to the surface.

Absorption of Carbohydrates. After the digestive process is complete, the carbohydrates of the food are in the form of monosaccharides: glucose, fructose, and galactose. These monosaccharides are absorbed directly into the blood stream. Some absorption into the lymph undoubtedly occurs. During absorption they are combined with phosphoric acid to form hexose phosphates. Before entering the blood stream the hexose phosphates are apparently hydrolyzed, since hexoses are found in the blood rather than hexose phosphates. The blood carries these hexoses to the liver, where they are converted into **glycogen,** in which form they are stored. It is an interesting fact that any hexose monosaccharide absorbed is converted into glycogen. Glycogen is also stored in muscles.

Undigested disaccharides are not absorbed. Lactose is less easily digested than maltose and sucrose and hence may find its way to the lower regions of the small intestine before digestion takes place. Some may even reach the large intestine, where it may serve as food for intestinal organisms. Certain lactose-fermenting microorganisms, which are said to be highly beneficial, may be encouraged to grow in the large intestine by eating large quantities of lactose.

Absorption of Fats. There are two theories regarding what happens during the digestion and absorption of fats, namely, the lipolytic theory and the partition theory.

Lipolytic Theory. The lipolytic theory assumes that during digestion fats are completely hydrolyzed to glycerol and fatty acids. These are absorbed into the intestinal mucosa where they are resynthesized into fat. About 70 per cent of this fat finds its way into the lymph and 30 per cent into the blood stream. The lymph containing fat appears milky and is called **chyle.**

Since glycerol is water-soluble, there is no difficulty in explaining its absorption. Fatty acids, however, are insoluble in water, and hence there must be another explanation for their absorption. Fatty acids are soluble in the presence of bile salts. It is believed that after digestion the fatty acids unite with the bile salts to form a complex, which is soluble and passes through the lining of the villi. As soon as absorption takes place, this complex breaks down, the fatty acids liberated form fat, and the bile salt is eventually carried to the liver, where it is resecreted in the bile.

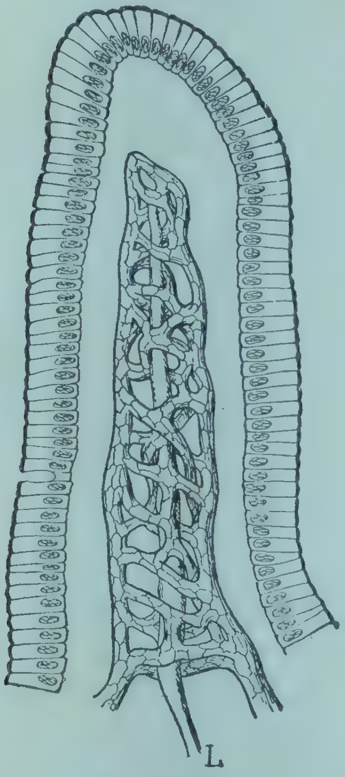


FIG. 19. The structure of a villus. On the outside are the lining cells of the intestine. Within is a network of capillaries in the center of which is a lymphatic, *L.* The loose tissue within the villus is omitted for simplicity. From *Nutritional Physiology* by Stiles. Courtesy of W. B. Saunders Co.

It is believed that phospholipids, such as lecithin, are involved in the resynthesis of fat from glycerol and fatty acids in the intestinal mucosa. Lecithin reacts with a molecule of fatty acid to form a fat, the fatty acid replacing the phosphoric acid-choline complex of lecithin. The phosphoric acid-choline complex next unites with glycerol and fatty acids to form more lecithin, and the process is repeated.

The villi through a process of contraction force the chyle into the larger lymphatics, and chyle finally enters the blood stream at the juncture of the subclavian and jugular veins under the left shoulder by way of the thoracic duct. The blood carries the fat to the tissues of the body, where some is oxidized and the rest is stored as fat.

Partition Theory. This theory, advanced by Frazer, assumes that fats are not completely hydrolyzed during digestion. Those molecules that are hydrolyzed are only partially broken down into mono- and diglycerides and some fatty acids. Some of these glycerides and fatty acids form a complex with the bile salts which are very powerful emulsifying agents. Under their influence undigested fat together with mono- and diglycerides are emulsified and absorbed directly into the lymph. The fatty acids are absorbed into the blood as a fatty acid-bile salt complex and carried to the liver.

Absorption of Sterols. The only sterol found in foods that is absorbed by the human intestine is cholesterol, the sterol found in animal fats. It is absorbed into the lymph in combination with the bile salts and also as cholesterol esters. Plant sterols are not absorbed. The D vitamins which are sterol derivatives are absorbed. This is also true of certain sex and adrenal hormones used in medicine. Cortisone acetate, the hormone preparation used in the treatment of rheumatoid arthritis, is about as effective when taken by mouth as when injected intramuscularly.

Absorption of Proteins. Proteins are absorbed as amino acids directly into the blood stream. After a protein meal the amount of amino acids in the blood increases, especially in the red corpuscles. The blood carries the amino acids to the tissues, where they become part of a metabolic pool from which body proteins are synthesized. Eventually amino acids are oxidized, producing energy.

There is some evidence that large molecules, such as polypeptides or peptones, may be absorbed under certain conditions. If undigested or partly digested proteins are repeatedly injected into the blood or tissue of an animal, the animal may receive a severe shock or even die after an injection of the same protein later on. Shock or death immediately after the administration of diphtheria antitoxin is usually due to the fact that the patient is sensitive to the proteins of the horse serum which contains the antitoxin. If the absorptive mechanism of a person allows partly hydrolyzed proteins to be absorbed, similar symptoms may develop upon the eating of specific proteins. Many conditions, such as asthma, eczema, and hay fever, are known to be aggravated by certain protein foods. It is believed that in these cases certain protein foods are absorbed in a partly digested form. After absorption they behave just as foreign proteins do when injected directly into the blood or tissues. This peculiar behavior of some individuals toward certain protein foods is interpreted as meaning that larger molecules than amino acids may be absorbed.

Absorption of Salts. In addition to the organic food materials, inorganic salts are also absorbed through the intestinal wall. However, not all inorganic ions are absorbed with equal ease. Sodium, potassium, and chloride ions are absorbed readily, but magnesium and sulfate ions are absorbed with difficulty. The laxative action of Epsom salt, which is the common name for magnesium sulfate, is due to the fact that magnesium sulfate is slowly absorbed and hence withdraws water from the body into the intestine, thus liquefying the feces. The absorption of calcium and phosphorus is aided by the administration of vitamin D, the antirachitic vitamin.

Mechanism of Absorption. The exact mechanism by which the simple food molecules are absorbed is not known. Undoubtedly such phenomena as osmosis and Donnan equilibrium play important parts, but they do not explain everything. There is some evidence that the white blood corpuscles, which are numerous in the intestinal mucosa, actually pass through the intestinal wall, become loaded with food, and carry it back into the blood and lymph. Some deep-seated chemical attraction, as yet undetermined, between the protoplasm of the tissue cells and food, may prove to be the real explanation of how foods are absorbed.

Feces. As the contents of the small intestine enter the large intestine, they are semifluid and consist largely of undigested food residues and the remains of the digestive juices. There is very little further digestion in the large intestine. Much of the water of the intestinal contents is absorbed here. The contents of the large intestine are called the **feces.** About one-fourth of dried feces is bacteria. In addition to food residues the feces contain bile salts, bile pigments, sterols, mucin, and inorganic salts.

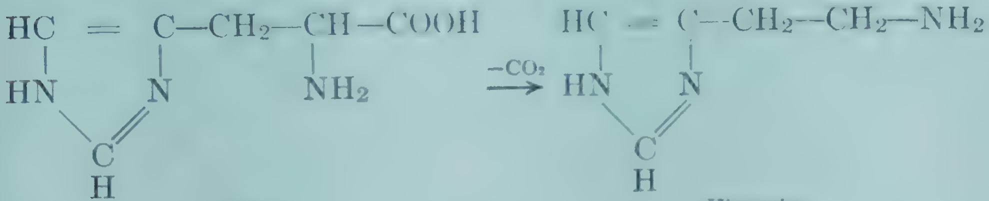
The diet only partially determines the character of the feces. During starvation the quantity of feces is greatly reduced, but still they are produced and are not greatly different from what they normally are. This fact indicates that the feces are produced mainly by an excretion of the digestive tract. Foods which are bulky and contain much indigestible matter, such as cellulose, increase the volume of feces. Upon an ordinary mixed diet, from 25 to 50 grams of fecal dry matter is produced per day.

Intestinal Putrefaction. It has been estimated that about one-fourth of the fecal matter is microorganisms. These microorganisms live on the organic matter present and are responsible for many products of metabolism, some of which are beneficial, and others of which may be harmful, to man.

Among the products which may be utilized by man are such substances as ethyl alcohol and lactic, acetic, butyric, and succinic acids. In herbivorous animals it has been shown that cellulose may be converted by bacteria in the digestive tract into simpler compounds which are utilizable by the animal. In cows certain bacteria present in the rumen have the ability to synthesize vitamin B_1. That this capacity for synthesis may be an important source of this vitamin for cows is suggested by the fact that cows can live on a diet lacking vitamin B_1. There is at present considerable evidence to indicate that intestinal microorganisms are an important source of several vitamins in man and other animals. It may be stated that in general the beneficial or harmless products of bacterial action in the digestive tract are the result of the action of bacteria on carbohydrates.

The harmful products of intestinal putrefaction are the result of bacterial action on amino acids derived from proteins. The number of possible physiologically active products which may be derived from amino acids is so large that not all of them can be discussed here; only a few typical examples will be mentioned.

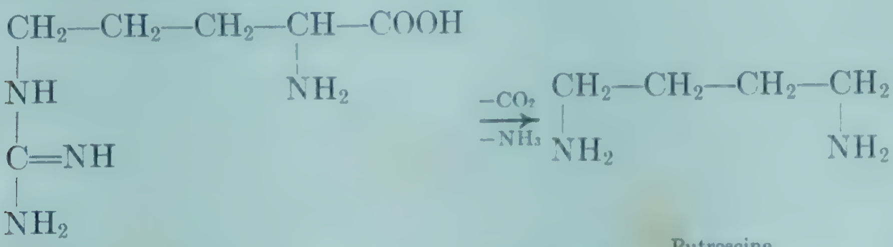
If carbon dioxide is removed from the carboxyl group of an amino acid, an amine results. Many of these amines are toxic, and others have marked physiological properties. An example of a physiologically active amine is **histamine**, which may be formed from histidine thus:



Histidine

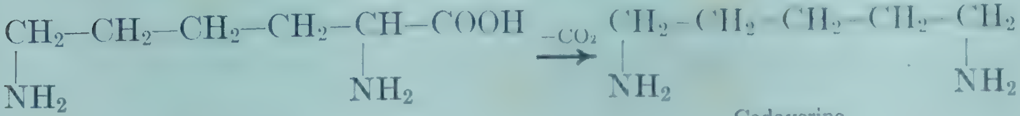
Histamine

Arginine and lysine give rise to **putrescine** and **cadaverine**, respectively. These amines have been isolated from decaying flesh and are relatively harmless.



Arginine

Putrescine

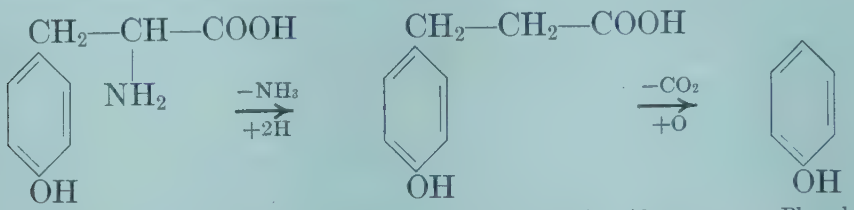


$$CH_2{-}CH_2{-}CH_2{-}CH_2{-}CH(NH_2){-}COOH \xrightarrow{-CO_2} CH_2(NH_2){-}CH_2{-}CH_2{-}CH_2{-}CH_2(NH_2)$$

Lysine

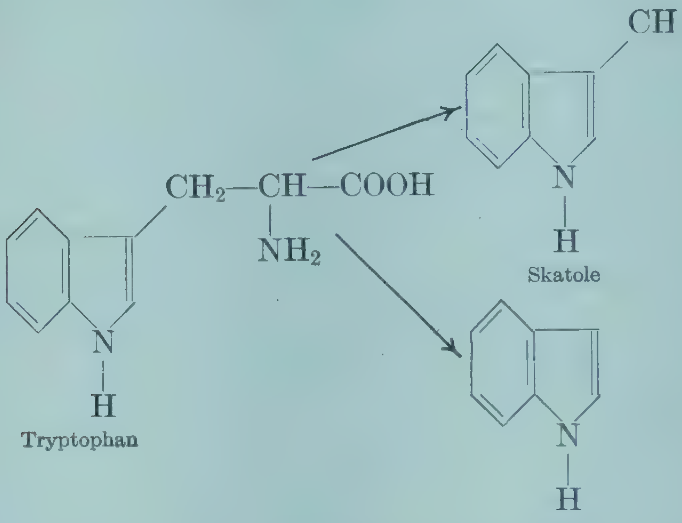
Cadaverine

Another type of change which may occur in an amino acid because of intestinal putrefaction involves the removal of the amino group and the production of an acid. In the aromatic amino acids the side chain may be further oxidized, leaving toxic products. Examples of this type of change are the production of **phenol** from tyrosine, and **indole** and **skatole** from tryptophan. Phenol may arise from tyrosine thus:



Tyrosine p-Hydroxyphenylpropionic acid Phenol

By a similar process skatole and indole can originate from tryptophan. The following diagram does not indicate the intermediate steps in this reaction.



Skatole

Tryptophan

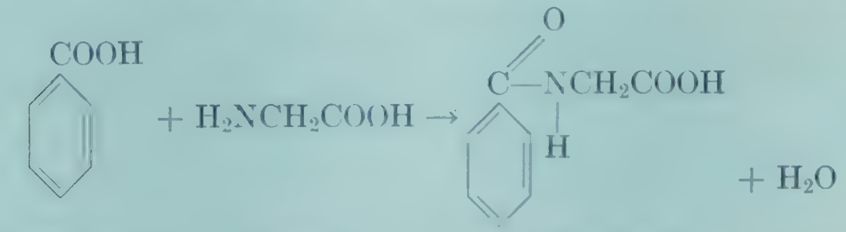
Indole

Indole and skatole are the compounds responsible for the characteristic odor of feces. When they are absorbed into the blood in sufficient quantities, they give the breath a fecal odor, sometimes spoken of as halitosis.

Another toxic product resulting from the intestinal putrefaction of proteins is hydrogen sulfide, derived from the amino acid cystine.

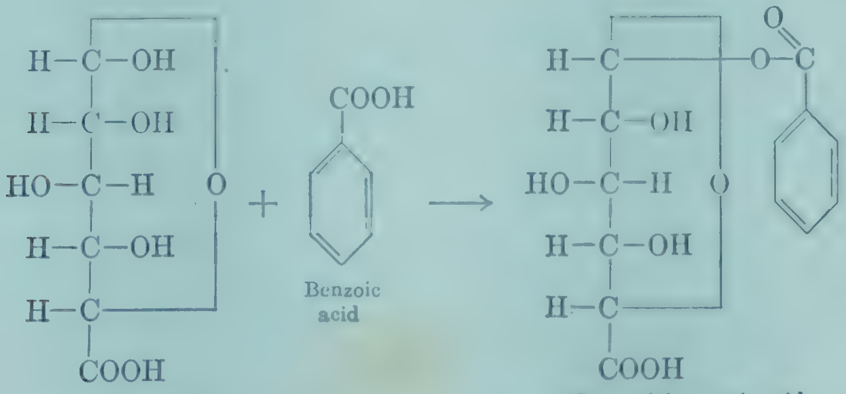
The most important harmful effect of bacteria is that many of them produce toxins, often referred to as **ptomaines**, which, sometimes, in food poisoning, are sufficiently potent to cause death. It is quite possible that even bacteria ordinarily considered harmless produce mild toxins which may account for many minor ailments, such as headaches. It has been shown that many bacteria that produce toxins on media rich in proteins do not do so on media rich in carbohydrates. For this reason a low-protein diet and one containing a sugar such as lactose, which is not easily digested and which therefore reaches the lower bowel, may be beneficial in preventing undesirable intestinal putrefaction.

Detoxication. From what has been said, it might be inferred that the products of intestinal putrefaction are a constant menace to health. This would possibly be true if the body did not have excellent defense mechanisms against these toxic products. Under normal conditions the body can take care of the small amounts of toxic substances absorbed from the intestine. Perhaps the most interesting method is that known as **protective synthesis,** in which the toxic substance is combined with some harmless compound so that the toxic compound is less poisonous and more easily eliminated by the kidney. For example, benzoic acid may unite with glycine to form hippuric acid thus:



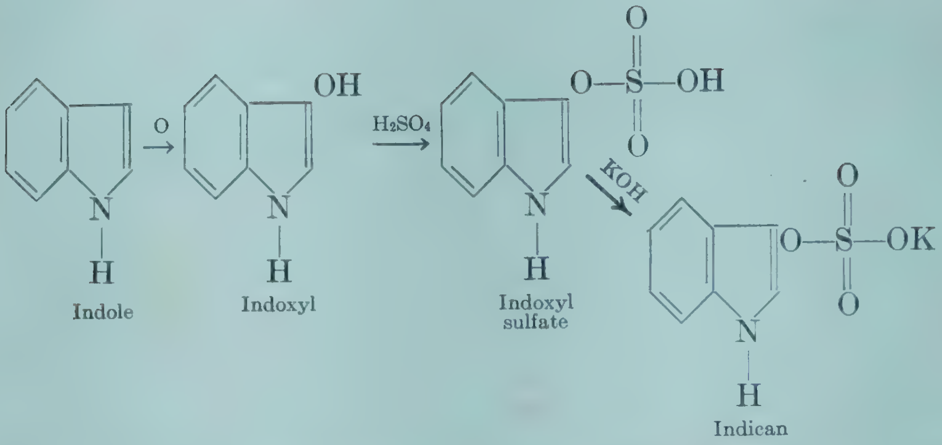
Benzoic acid Glycine Hippuric acid

Benzoic acid may also unite with glucuronic acid to form an ester which is no longer toxic and which is easily eliminated. Phenol may unite with sulfuric acid to form a sulfate or with glucuronic acid to form a glycoside-like complex.



Glucuronic acid 1-Benzoylglucuronic acid

Indole may be oxidized to the hydroxy derivative and conjugated with sulfuric acid. The potassium salt of this derivative is called **indican.** Its presence in the urine may be determined, and the quantity present is said to be a measure of intestinal putrefaction.



Other types of detoxication involve reduction, hydrolysis, or the oxidation of the toxic product to harmless products. Other examples of protective synthesis could be given if space permitted.

REVIEW QUESTIONS

1. Define absorption. In what part of the digestive tract does most absorption occur?
2. Describe the structure of a villus.
3. What is lymph?
4. Discuss the absorption of carbohydrates, fats, proteins, and sterols. In what chemical form is each absorbed? Which foods are absorbed directly into the blood stream, and which into the lymph?
5. Give one reason why Epsom salt is a laxative.
6. Discuss the mechanism of absorption.
7. Discuss the nature and origin of feces. How does diet affect the amount and nature of feces?
8. Discuss intestinal putrefaction.
9. How may histamine, putrescine, cadaverine, phenol, skatole, and indole originate in the intestine?
10. Name two important methods by which the body removes toxic products of putrefaction.
11. What is meant by protective synthesis? Give an example.

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12

Carbohydrate Metabolism

Metabolism. During the process of digestion the complex food molecules are broken down into the simple units of which they are composed. These simple molecules pass through the intestinal lining, either directly into the blood stream or indirectly through the lymph. By the blood they are carried to all the tissues of the body, where they either are used for building new or repairing worn-out body tissue or are oxidized for energy. Any excess may be stored in the form of glycogen, fat, or protein for future use. The chemical changes which absorbed food molecules undergo after absorption are known as metabolism.

Until recently, largely because of the work of Folin, it was thought that there were two distinct types of metabolism, namely, **endogenous** and **exogenous.** If a food molecule became a part of the body tissue, it was said to go the endogenous route of metabolism. Food molecules which never became a part of body tissue but were oxidized for energy were said to go the exogenous route. These two routes of metabolism were thought to be distinct and independent of each other.

The building-up phase of endogenous metabolism was called **anabolism,** and the breakdown of tissue was referred to as **catabolism.** The breakdown of other than tissue molecules was also referred to as catabolism.

Work by Schoenheimer and others, in which molecules were labeled by introducing isotopic or heavy hydrogen, nitrogen, carbon, sulfur, or radioactive phosphorus atoms, has led to the conclusion that there is no sharp dividing line between endogenous and exogenous metabolism. In fact, these two types of metabolism are so intimately associated that they must be considered one. It has been shown that labeled food molecules are continually entering and leaving tissue molecules, that groups from one molecule are passed on to other molecules, and that there is a constant change of one molecule into another. In fact, living matter appears to be in a constant state of flux.

Carbohydrates are used by the body mainly as a source of energy. However, it should be borne in mind that they are found in some of the most important compounds of the body, such as the nucleoproteins of the cell nuclei, the galactosides of the brain, and the glycoproteins of tendons, cartilages, and the mucin of the saliva.

Glycogen. During the digestion of carbohydrate foods the monosaccharides of importance which result are glucose, fructose, and galactose. They are absorbed mainly into the blood stream and are carried by the portal circulation to the liver. In the portal circulation all three of these monosaccharides may be found, and the concentration of sugar after a meal may be twice as high in the portal as in the general circulation. In the general circulation the sugar in the blood is glucose, which is present normally to the extent of about 0.07 to 0.10 per cent or, as usually stated, 70 to 100 mg. per 100 ml.

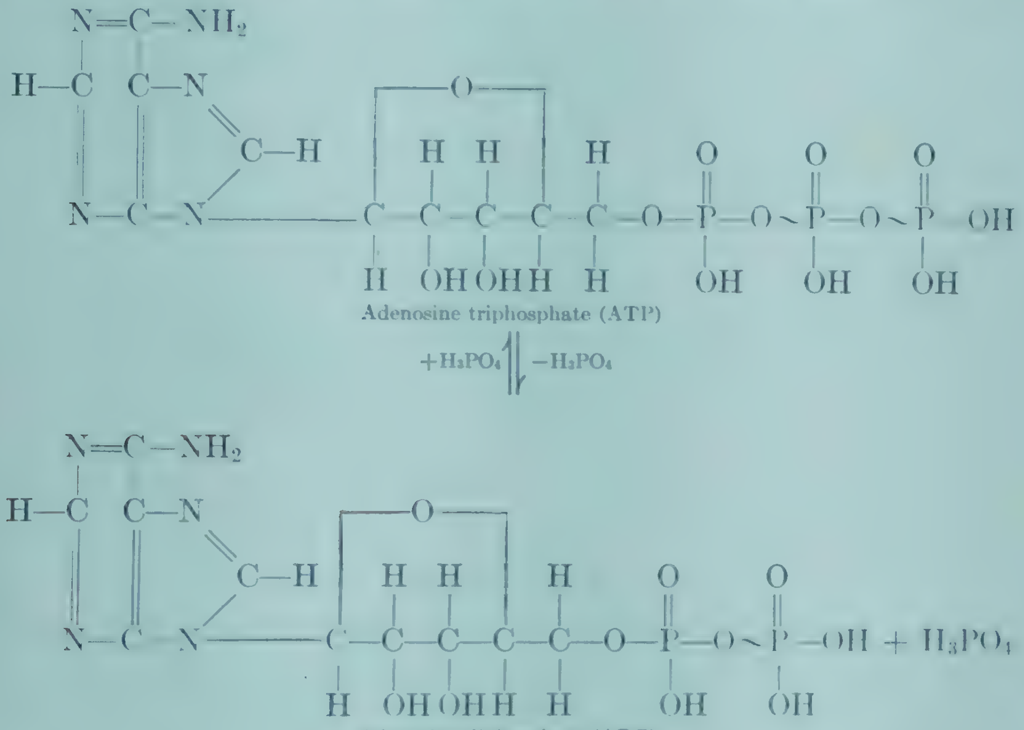
In the liver, glucose, fructose, and galactose are converted into glycogen, sometimes called animal starch, and stored there for future use. Another storage of glycogen is in the muscles, the glycogen being formed from the glucose in the blood. The liver may store 10 to 15 per cent by weight of glycogen, and the muscles 2 per cent. The glycogen stored in the liver serves as a reservoir from which glucose may be drawn to maintain a rather constant concentration in the blood. The glycogen stored in the muscles is not reconverted into glucose but into a glucose phosphate which is metabolized directly. The transformation of sugars into glycogen is called **glycogenesis.** The reverse process, the transformation of glycogen into glucose, is called **glycogenolysis.**

If large quantities of sugar are eaten, the body is unable to convert it all to glycogen, and the concentration in the blood increases. When the concentration in the blood reaches 160 to 180 mg. per 100 ml., sugar appears in the urine. The point at which sugar appears in the urine is called the **sugar threshold.**

The glucose in the blood may originate from other sources than the carbohydrate in the food. If more protein is eaten than is required by the body, certain of the amino acids may be converted into glucose. About 60 per cent of the amino acids in protein are capable of forming glucose in the body. Since the glycerol of fats may form glucose in the body, and since fats are composed of about 10 per cent glycerol, 10 per cent of fat may be so converted. Apparently the fatty acids of a fat cannot be converted directly into glucose. However, it is possible that certain intermediates in fatty acid metabolism may be involved in carbohydrate synthesis.

As will be pointed out later, lactic acid, which is a product of glucose metabolism, may be reconverted into glycogen in the liver. The synthesis of glucose from noncarbohydrate materials is called **gluconeogenesis.**

Glycogenesis. Before glucose is converted into glycogen it first reacts with phosphoric acid to form glucose-6-phosphate. The phosphorylation is brought about by an enzyme called phosphorylase (hexokinase) and a coenzyme called adenosine triphosphate, usually abbreviated to ATP. ATP furnishes the phosphoric acid for the phosphorylation and becomes adenosine diphosphate (ADP). In the formulas for ATP and ADP given here certain bonds to P are represented by the symbol ~. This kind of bond is known as a high-energy phosphate bond, and its significance will be discussed later.



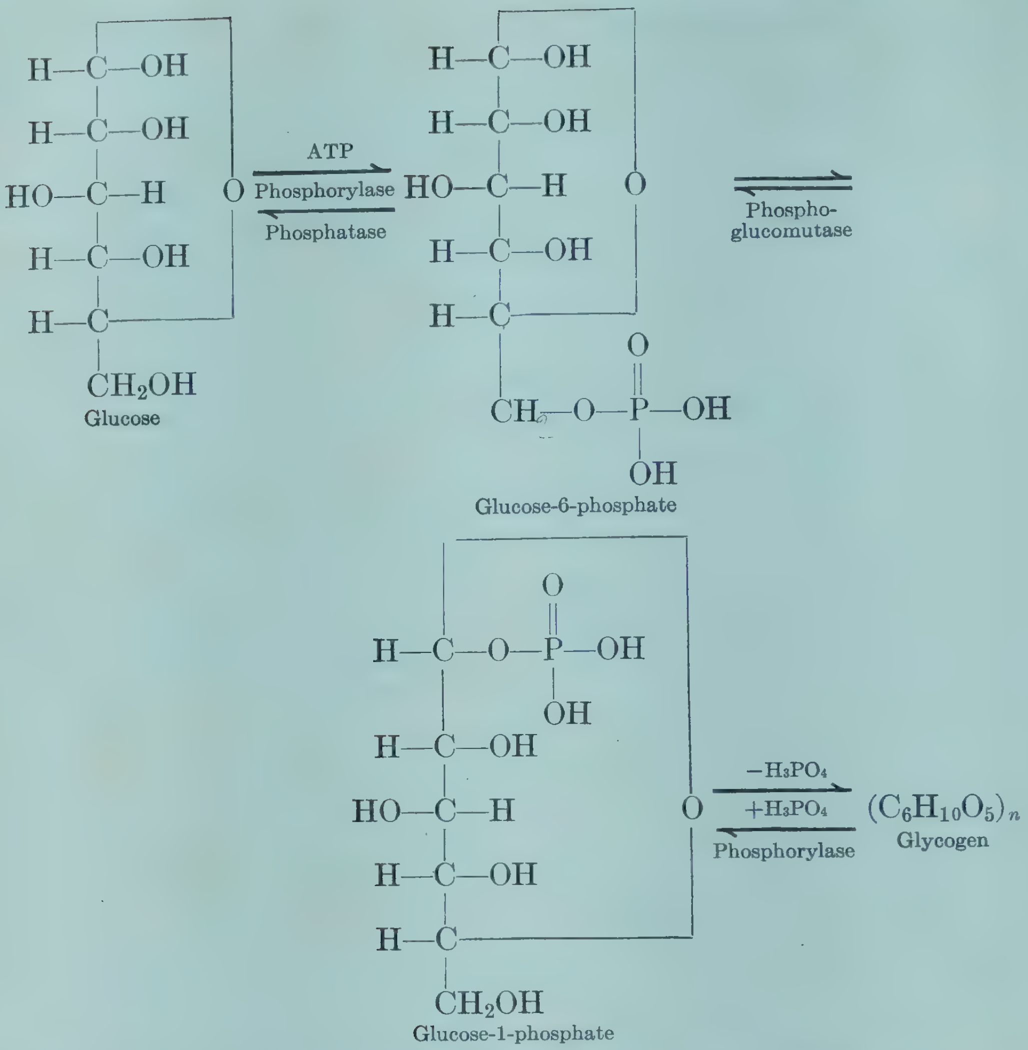
Adenosine triphosphate (ATP)

$+H_3PO_4$ ⇅ $-H_3PO_4$

Adenosine diphosphate (ADP)

After glucose-6-phosphate is formed, the enzyme phosphoglucomutase transfers the phosphate group from carbon 6 to carbon 1, producing glucose-1-phosphate. Finally, phosphorylase converts many molecules of glucose-1-phosphate into glycogen and phosphoric acid.

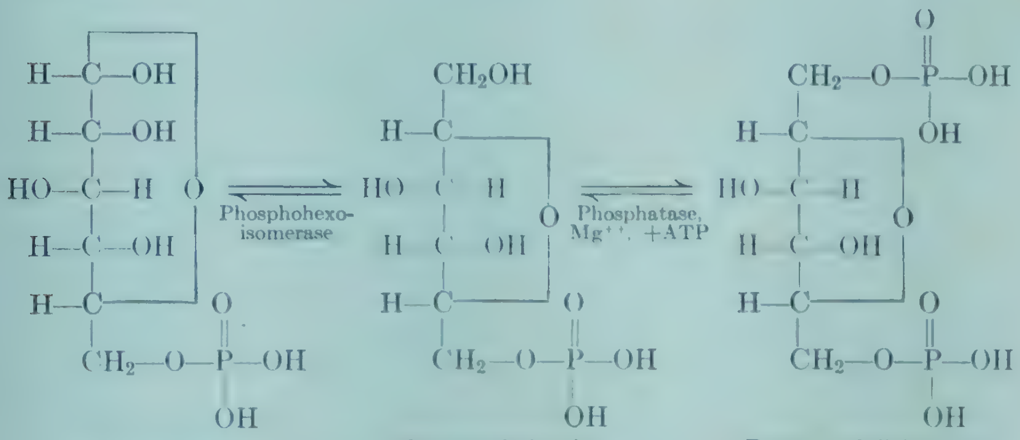
Glycogenolysis. When blood-glucose values become low, glycogen in the liver is reconverted into glucose to increase the supply in the blood. In this process the reactions just discussed are reversed. Glycogen, in the presence of inorganic phosphate and phosphorylase, is converted into glucose-1-phosphate. This in turn is converted into glucose-6-phosphate by phosphoglucomutase. The glucose-6-phosphate is hydrolyzed to glucose and phosphoric acid by a phosphatase present in the liver. A similar series of reactions occurs when muscle glycogen


ATP
Phosphorylase
Phosphatase
Glucose
Glucose-6-phosphate
Phospho-
glucomutase
−H₃PO₄
+H₃PO₄
Phosphorylase
(C₆H₁₀O₅)ₙ
Glycogen
Glucose-1-phosphate

hydrolyzes. There is, however, this difference, the reaction stops at the glucose-6-phosphate stage. There is no phosphatase present in the muscle to convert glucose-6-phosphate into glucose and phosphoric acid.

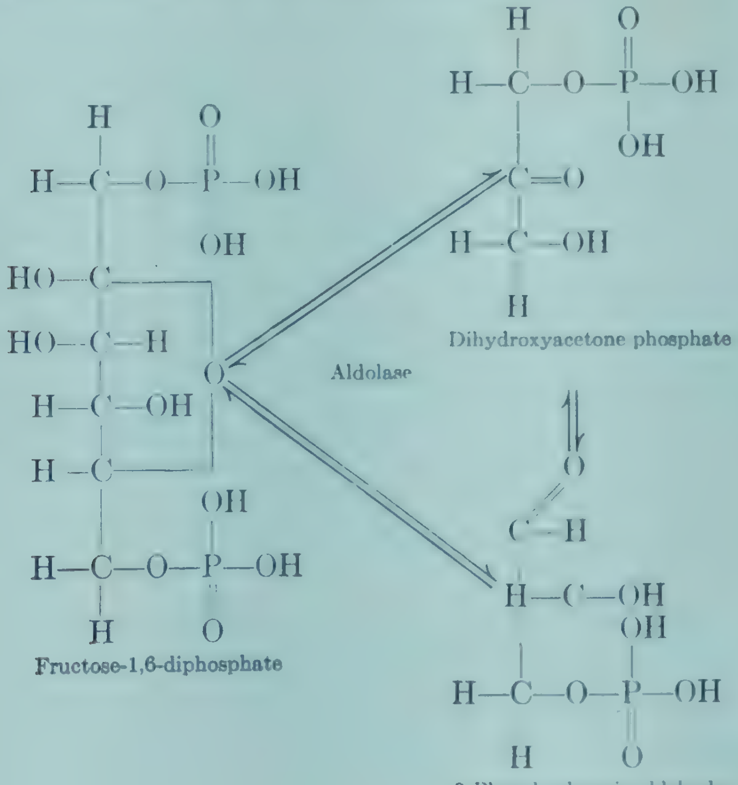
Utilization of Glucose for Energy. The breakdown of glucose in muscle and other tissues into carbon dioxide and water for the production of energy takes place in two stages, one requiring no oxygen, known as the **anaerobic stage** or **glucolysis,** and the other requiring oxygen, known as the **aerobic stage.** Glucolysis involves the breakdown of glucose to pyruvic and lactic acids; the aerobic stage, the breakdown of lactic acid to carbon dioxide and water. Glucolysis in muscle resembles quite closely the fermentation of glucose by yeast with this important difference. In muscle the end product is pyruvic or lactic acid, whereas in yeast fermentation the end products are ethyl alcohol and carbon dioxide.

Glucolysis. We have shown above how glycogen in the muscle breaks down forming glucose-1-phosphate and how this is converted into glucose-6-phosphate. In the next step glucose-6-phosphate is converted into fructose-6-phosphate by the enzyme phosphohexoisomerase. Fructose-6-phosphate is next phosphorylated to form fructose-1,6-diphosphate by the enzyme phosphatase in the presence of Mg^{++} and ATP.



Glucose-6-phosphate Fructose-6-phosphate Fructose-1,6-diphosphate

The next step involves the splitting of the fructose-1,6-diphosphate by the enzyme aldolase into two triose monophosphates, thus:

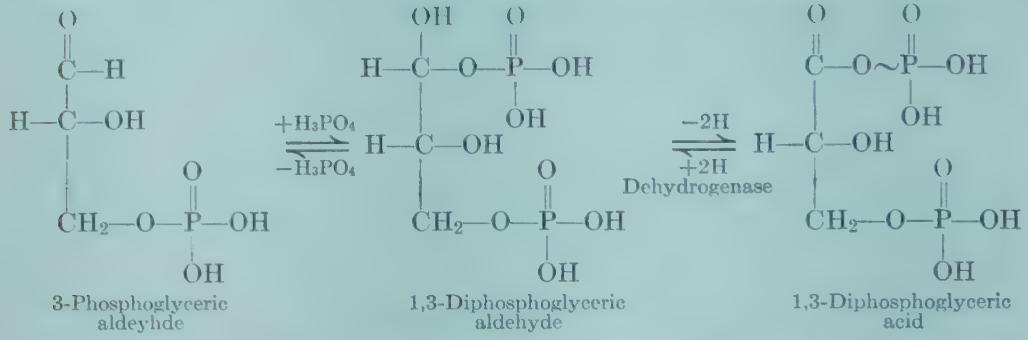


Dihydroxyacetone phosphate

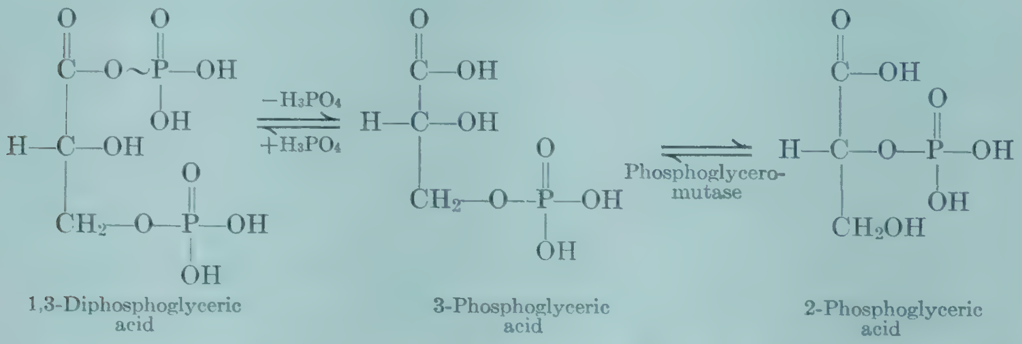
Fructose-1,6-diphosphate

3-Phosphoglyceric aldehyde

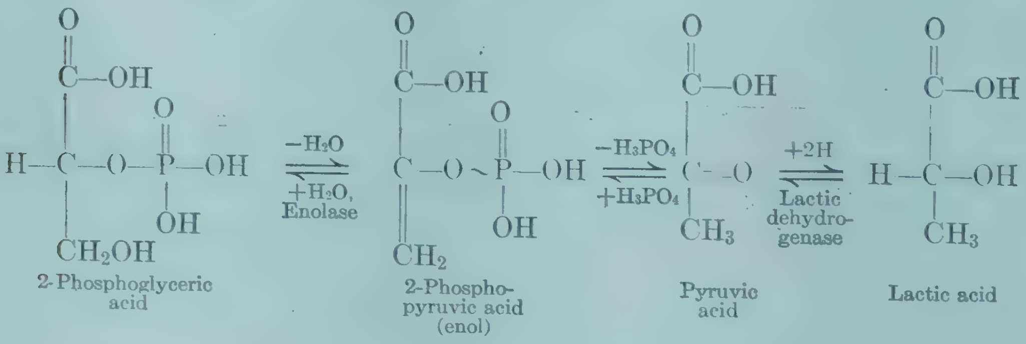
The two triose monophosphates form an equilibrium mixture. Next 3-phosphoglyceric aldehyde adds another phosphate group to form 1,3-diphosphoglyceric aldehyde. This in the presence of a dehydrogenase and coenzyme I gives 1,3-diphosphoglyceric acid.


+H₃PO₄
−H₃PO₄
−2H
+2H
Dehydrogenase
3-Phosphoglyceric aldeyhde
1,3-Diphosphoglyceric aldehyde
1,3-Diphosphoglyceric acid

It should be noted that one of the phosphate linkages in 1,3-diphosphoglyceric acid is of the high-energy variety. The next step in the reaction involves the cleavage of this bond with the formation of 3-phosphoglyceric acid and the releasing of energy. The phosphoric acid is next transferred from the 3 to the 2 position by the enzyme phosphoglyceromutase so that 2-phosphoglyceric acid is formed.


−H₃PO₄
+H₃PO₄
Phosphoglyceromutase
1,3-Diphosphoglyceric acid
3-Phosphoglyceric acid
2-Phosphoglyceric acid

Next 2-phosphoglyceric acid is converted into the enol of 2-phosphopyruvic acid by the enzyme enolase. This molecule contains a high-energy phosphate bond. 2-Phosphopyruvic acid then loses its phosphoric acid releasing energy and forming pyruvic acid, which finally is reduced by lactic acid dehydrogenase to form lactic acid.


−H₂O
+H₂O,
Enolase
−H₃PO₄
+H₃PO₄
+2H
Lactic dehydrogenase
2-Phosphoglyceric acid
2-Phosphopyruvic acid (enol)
Pyruvic acid
Lactic acid

The steps in the conversion of glycogen into lactic acid may be summarized as follows:

Glycogen
⇅
Glucose-1-phosphate
⇅
Glucose-6-phosphate $\xleftarrow[\text{Hexokinase}]{\text{ATP}}$ Glucose
⇅
Fructose-6-phosphate
⇅
Fructose-1,6-diphosphate
⇅
3-Phosphoglyceric aldehyde ⇌ Dihydroxyacetone phosphate
⇅
1,3-Diphosphoglyceric aldehyde
⇅
1,3-Diphosphoglyceric acid
⇅
3-Phosphoglyceric acid
⇅
2-Phosphoglyceric acid
⇅
2-Phosphopyruvic acid (enol)
⇅
Pyruvic acid
⇅
Lactic acid

In yeast fermentation the chemical changes are probably similar to those just discussed, except that, when the pyruvic acid stage is reached, decarboxylation takes place with the formation of **acetaldehyde** instead of reduction with the formation of lactic acid. The acetaldehyde is then reduced to **ethyl alcohol.**

THE LACTIC ACID CYCLE. There still remains the question of what happens to the lactic acid produced in the muscle. It is believed that the acid passes into the blood stream and is transported to the liver, where it is converted into glycogen. Some finds its way into the urine. About 20 per cent is oxidized to carbon dioxide and water. Lactic acid is not converted into muscle glycogen. Muscle glycogen comes only from the glucose of the blood. Figure 20 shows diagrammatically the lactic acid cycle.

In the presence of an adequate supply of oxygen no appreciable amount of lactic acid can be detected in muscle. For this reason it is sometimes thought that lactic acid is not a normal intermediate in carbohydrate metabolism, but that pyruvic acid is directly oxidized to carbon dioxide and water.

Aerobic Stage. We have just considered the reactions involved in the conversion of glycogen into pyruvic and lactic acids. We next consider how these acids are oxidized to carbon dioxide and water. The metabolic pathway by which this occurs is known as the **Krebs tricarboxylic acid cycle.** Figure 21 shows the steps in the Krebs cycle.

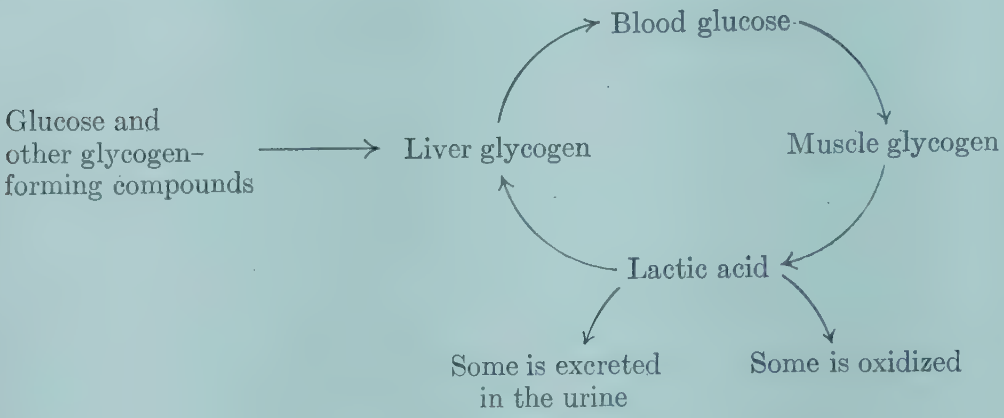

Blood glucose
Glucose and other glycogen-forming compounds
Liver glycogen
Muscle glycogen
Lactic acid
Some is excreted in the urine
Some is oxidized

FIG. 20. The lactic acid cycle.

According to Krebs, lactic acid is oxidized to pyruvic acid which loses carbon dioxide and hydrogen to form an acetyl group. The acetyl group reacts with oxaloacetic acid to form citric acid which loses a molecule of water to form *cis*-aconitic acid. *cis*-Aconitic acid next adds a molecule of water to form isocitric acid. Isocitric acid is next oxidized to oxalosuccinic acid which loses carbon dioxide to form α-ketoglutaric acid. This is decarboxylated and oxidized to form succinic acid. Succinic acid next loses two hydrogen atoms forming fumaric acid, which adds a molecule of water to form malic acid. Finally malic acid loses two hydrogen atoms to form oxaloacetic acid, and the cycle is completed. Oxaloacetic acid may lose carbon dioxide to form pyruvic acid, which would again enter into the cycle. The net result of one turn of the cycle is the oxidation of one molecule of pyruvic acid with the formation of three molecules of carbon dioxide and three molecules of water. Each of the above reactions is catalyzed by an appropriate enzyme. All of the above reactions are reversible except the decarboxylation of pyruvic and α-ketoglutaric acids. However, it should be pointed out that, as the cycle proceeds in a clockwise direction, oxaloacetic acid is formed which on decarboxylation gives pyruvic acid. Thus the cycle which starts with pyruvic acid is in a sense reversible.

It should be noted that other molecules besides carbohydrates may be oxidized via the Krebs cycle. It is not difficult to see how glycerol

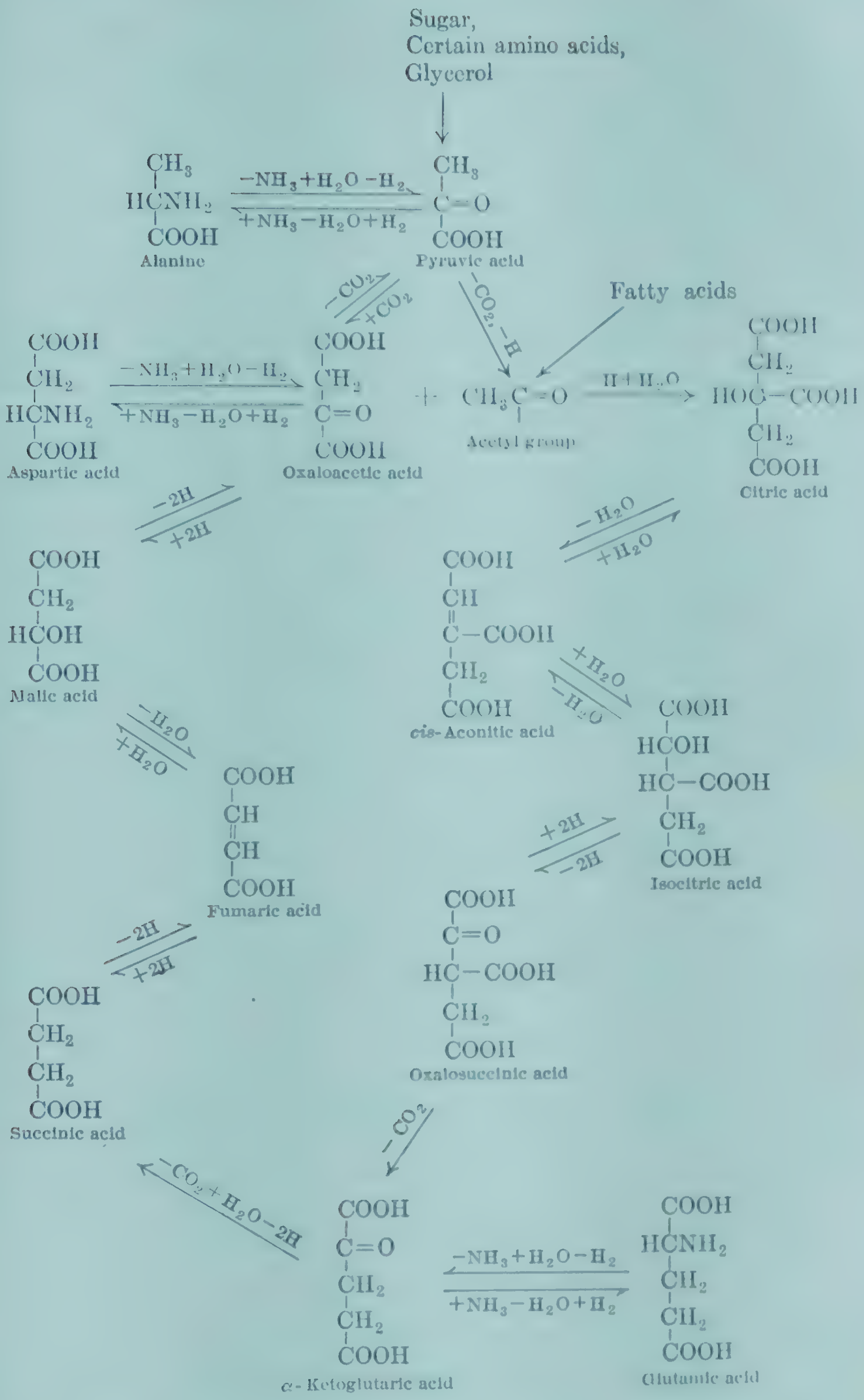

Sugar,
Certain amino acids,
Glycerol
Alanine
$-NH_3+H_2O-H_2$
$+NH_3-H_2O+H_2$
Pyruvic acid
$-CO_2$
$+CO_2$
$-CO_2, -H$
Fatty acids
Aspartic acid
$-NH_3+H_2O-H_2$
$+NH_3-H_2O+H_2$
Oxaloacetic acid
+
Acetyl group
$H+H_2O$
Citric acid
−2H
+2H
$-H_2O$
$+H_2O$
Malic acid
cis-Aconitic acid
$+H_2O$
$-H_2O$
$-H_2O$
$+H_2O$
Fumaric acid
+2H
−2H
Isocitric acid
−2H
+2H
Oxalosuccinic acid
Succinic acid
$-CO_2$
$-CO_2+H_2O-2H$
α-Ketoglutaric acid
$-NH_3+H_2O-H_2$
$+NH_3-H_2O+H_2$
Glutamic acid

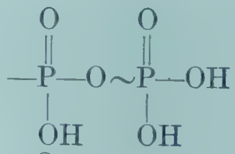
Fig. 21. Krebs tricarboxylic acid cycle in carbohydrate metabolism.

might be converted into pyruvic acid via the triose phosphates. Fatty acids may produce acetyl groups during their metabolism. Alanine, aspartic acid, and glutamic acid give, respectively, pyruvic, oxaloacetic, and α-ketoglutaric acids on oxidation. All of these molecules would fit into the Krebs cycle. The Krebs cycle, therefore, should not be considered an exclusive pathway for carbohydrate metabolism alone but as serving as a pathway for the metabolism of fats and proteins also.

High-Energy Phosphate Bonds. It is evident from the foregoing discussion of the anaerobic stage of carbohydrate metabolism that phosphoric acid is an important factor in the process. All of the intermediate compounds from glycogen to pyruvic acid are phosphates. There are two classes of phosphates based on the amount of energy which they release on hydrolysis. One contains a low- and the other a high-energy phosphate bond; the latter is represented by the symbol ∼. Low-energy phosphate bonds release about 2000 calories whereas high-energy phosphate bonds release about 12,000 calories, per mole, when hydrolyzed. An example of a low-energy phosphate bond is the ester linkage of phosphoric acid through an alcoholic hydroxyl group. Such linkages occur in the hexose phosphates, triose phosphates, 2-phosphoglyceric acid, and 3-phosphoglyceric acid.

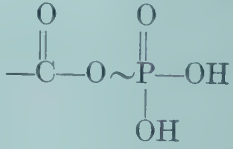
Four types of high-energy phosphate bonds are recognized:

(1) To another phosphate group:



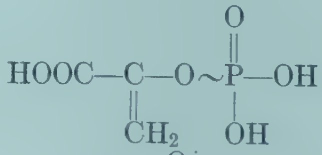
$$-\underset{\underset{\displaystyle OH}{|}}{\overset{\overset{\displaystyle O}{\|}}{P}}-O\sim\underset{\underset{\displaystyle OH}{|}}{\overset{\overset{\displaystyle O}{\|}}{P}}-OH$$

(2) To a carboxyl group:



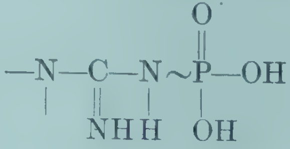
$$-\overset{\overset{\displaystyle O}{\|}}{C}-O\sim\underset{\underset{\displaystyle OH}{|}}{\overset{\overset{\displaystyle O}{\|}}{P}}-OH$$

(3) To an acidic enol group:



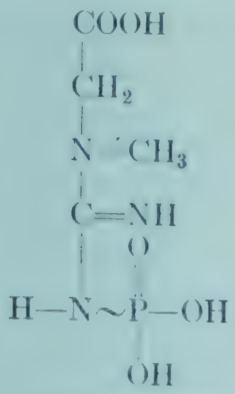
$$HOOC-\underset{\underset{\displaystyle CH_2}{\|}}{C}-O\sim\underset{\underset{\displaystyle OH}{|}}{\overset{\overset{\displaystyle O}{\|}}{P}}-OH$$

(4) To a guanidine group:



$$-\underset{|}{N}-\underset{\underset{\displaystyle NH}{\|}}{C}-\underset{\underset{\displaystyle H}{|}}{N}\sim\underset{\underset{\displaystyle OH}{|}}{\overset{\overset{\displaystyle O}{\|}}{P}}-OH$$

Examples of the above types of linkages are (1) ATP and ADP, (2) 1,3-diphosphoglyceric acid, (3) the enol of 2-phosphopyruvic acid, and (4) creatine phosphate. Creatine phosphate, an important compound found in the muscles, has the following formula:



Creatine phosphate

The Chemistry of Muscular Contraction. It is obvious that when muscles contract mechanical work is performed and energy is required. The question of where this energy comes from has interested physiologists from early days. Liebig assumed, without experimental proof, that since muscles are largely protein, the energy comes from the breakdown of muscle protein. Because of Liebig's eminence as a scientist, this view held for some time. In 1866 Fick and Wislicenus ascended a Swiss mountain and collected the urine produced during the ascent. On returning to their laboratory they analyzed this urine for nitrogen and found that it contained little more than would be expected if they had done a normal day's work. They concluded that since strenuous exercise did not increase nitrogen excretion, proteins could not be an important source of the energy produced. The present conception is that under normal conditions carbohydrates are the primary source of energy for muscular contraction. However, if carbohydrate reserves become too low, protein and fat may be used.

We shall now consider the chemistry involved in muscular contraction. There are two compounds in muscle that contain high-energy phosphate bonds, namely, ATP and creatine phosphate; the former is present in small amounts and the latter in relatively large amounts. The immediate source of energy for muscular contraction is ATP. When a muscle contracts ATP changes to ADP and phosphoric acid, and energy is released. This energy activates the muscle protein causing it to contract.

$$\text{ATP} + \text{Muscle protein} \longrightarrow \text{Contracted muscle} + \text{ADP} + H_3PO_4$$

Since muscles contain only small amounts of ATP, the supply must be replenished in order that a muscle may continue to contract. A large reserve of high-energy phosphate bonds exists in muscle in the form of creatine phosphate. This breaks down, and its high-energy phosphate is transferred to ADP to form ATP, which can be used to activate more muscle protein.

$$\text{Creatine phosphate} + \text{ADP} \rightleftharpoons \text{ATP} + \text{Creatine}$$

We next consider the origin of creatine phosphate. It is synthesized from creatine and ATP. The high-energy phosphate bond may come indirectly from 1,3-diphosphoglyceric acid or the enol of phosphopyruvic acid (both are intermediates in the anaerobic stage of carbohydrate metabolism), with ATP acting as an intermediate.

We finally discuss the ultimate source of the high-energy phosphate bonds. During the conversion of glycogen to pyruvic acid and in the oxidation of pyruvic acid to carbon dioxide and water energy is released. This energy is trapped in the high-energy phosphate bonds of ATP. The ATP may be used directly to stimulate muscles to contract, to synthesize 1,3-diphosphoglyceric acid and the enol of phosphopyruvic acid, or to form creatine phosphate. It may also be used to phosphorylate glucose and fructose in which case the high-energy phosphate bond is lost, the energy being absorbed in the reaction.

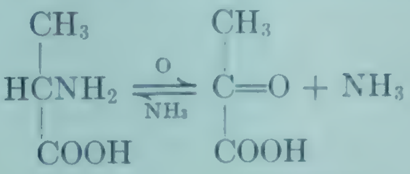
It should be pointed out that muscles can contract for a time in the absence of oxygen. The energy comes from that stored in high-energy phosphate bonds and from that arising from the conversion of glycogen to lactic acid, which is an anaerobic process. The accumulation of lactic acid stops the process. To start the process again, lactic acid is oxidized, furnishing energy to recharge the system. In many respects a muscle is like an electric motor run on a storage battery. Eventually the battery will be discharged, and the motor will stop until the battery is recharged. A muscle is not like a heat engine where fuel is burned before the engine will run.

Because muscles can contract anaerobically for a time; it is possible for a person to do more work for a short period than his oxygen intake would warrant. On such occasions he is said to go into **oxygen debt.** An athlete in a race may do work which would require the consumption of oxygen at the rate of 30 liters per minute, whereas he can breathe in only about 4 liters per minute. Thus he goes into oxygen debt which is repaid by heavy breathing for some time after the race is over.

Conversion of Carbohydrates to Fats. It is common knowledge that the consumption of large quantities of carbohydrates causes a person to become fat. It is therefore evident that the body has a mechanism for converting carbohydrates to fats. That such a conversion actually takes place may easily be demonstrated. Fats contain less oxygen than carbohydrates. When carbohydrates are converted into fats, the extra oxygen is used for oxidation purposes. Thus one consumes less oxygen from the air than when this conversion is not going on. When carbohydrates are being oxidized, the volume of oxygen consumed is equal to the volume of carbon dioxide produced. When carbohydrates are changing to fats, the volume of oxygen consumed is less than the volume of carbon dioxide produced. Since this high ratio of carbon dioxide production to oxygen consumption actually can be demonstrated, it is taken as proof that fat synthesis from carbohydrates actually occurs. More direct evidence of fat synthesis has been obtained from experiments on cows. After cows have been fed on a low-fat diet, more fat has been found in the milk than could possibly come from the fat in the food.

As far as the mechanism by which fats are synthesized from glucose is concerned, we must consider the synthesis of both glycerol and fatty acids. Glycerol could easily be formed from phosphoglyceric aldehyde, an intermediate product of glucose metabolism. The synthesis of long-chain fatty acids is not so simple. It is an interesting fact that all the longer-chained fatty acids found in nature have an even number of carbon atoms. Therefore it appears as though the fatty acids are synthesized from simple units containing two carbon atoms. Possibly acetaldehyde, which could originate from the decarboxylation of pyruvic acid, is the fundamental unit from which long-chain fatty acids arise. A familiar reaction in organic chemistry is the **aldol condensation,** in which two molecules of acetaldehyde unite to form aldol, which is the hydroxy derivative of butyric aldehyde. Aldol condenses with more acetaldehyde, increasing the length of the chain by two carbon atoms. The operation is continued until long-chain hydroxy aldehydes are produced. By oxidations and reductions the fatty acids are produced. By removing water from the hydroxy acids, an OH from one carbon atom and an H from a neighboring carbon atom, a double bond would result, thus accounting for unsaturated fatty acids in fats.

Amino Acid Synthesis from Carbohydrates. When we consider the metabolism of proteins, we shall find that one of the first steps in the metabolism of amino acids is the removal of the NH_2 group to form **NH_3** and a **ketone acid.** For alanine the reaction would be thus:



$$\begin{array}{c}CH_3\\ |\\ HCNH_2\\ |\\ COOH\end{array} \underset{NH_3}{\overset{O}{\rightleftharpoons}} \begin{array}{c}CH_3\\ |\\ C{=}O\\ |\\ COOH\end{array} + NH_3$$

Alanine Pyruvic acid

The ketone acid here is pyruvic acid, one of the products formed during the metabolism of glucose. Since this reaction is reversible, it is obvious that alanine may be synthesized from pyruvic acid and ammonia. In like manner glutamic and aspartic acids may arise from α-ketoglutaric and oxaloacetic acids, respectively. It is quite possible that several of the simpler amino acids can be synthesized in the body from products of carbohydrate metabolism and ammonia.

Carbohydrate Synthesis from Protein and Fat. We have just seen that fats and certain amino acids may be synthesized from carbohydrates in the animal body. There is very definite evidence that glucose may arise from protein and to some extent from fat. In diabetes, a disease in which the body has lost the power of oxidizing sugar, sugar is eliminated in large quantities through the urine. If a diabetic person is given no food, sugar still appears in the urine even after the glycogen reserves are used up. Under these conditions the nitrogen in the urine also increases, showing that body protein is being converted into sugar. In severe diabetes the ratio of glucose to nitrogen in the urine is 3.65:1. These figures mean that for every gram of nitrogen in the urine there are 3.65 grams of glucose. Since the factor for converting nitrogen into glucose is 6.25, these figures also mean that the ratio of nitrogen in the urine to protein metabolized is 3.65:6.25. In other words, 58.4 per cent of the protein metabolized has been converted into glucose. The ratio of glucose to nitrogen in the urine is known as the **G : N ratio** and is of great importance in the study of diabetes.

With regard to the mechanism by which proteins are converted into glucose in the body, it might be said that if an amino acid during its metabolism gives an intermediate which is identical with one formed during carbohydrate metabolism, such an intermediate should be metabolized as carbohydrates are. Since all reactions in glucose metabolism are essentially reversible, any amino acid which enters the pathway of glucose metabolism should be able to form glucose. Figure 21 indicates how alanine and aspartic and glutamic acids may enter the pathway of glucose metabolism via pyruvic, oxaloacetic, and α-ketoglutaric acids, respectively.

The conversion of fats to carbohydrates in the animal body is a question upon which physiologists differ. All agree that the glycerol part of the fat molecule may be converted into sugar. The glycerol is probably first changed to glyceric aldehyde by oxidation. This on phosphorylation could give 3-phosphoglyceric aldehyde which would enter the pathway of glucose metabolism and form glucose. There is a difference of opinion as to whether or not the fatty acids of a fat can be converted into glucose. Since acetic acid is an intermediate in fatty acid metabolism, it would appear that an acetyl group could enter the Krebs cycle and at least contribute to the formation of glucose.

Diabetes. Diabetes is a disease in which the body is unable to utilize sugar. It is characterized by the appearance of sugar in the urine. Normally the urine contains about 0.05 per cent of sugar, a quantity so small that Fehling's solution is not reduced by it. In diabetes as much as 10 per cent of sugar is frequently found in the urine.

The fact that large amounts of sugar are found in the urine does not necessarily mean that the subject has diabetes. If large quantities of sugar are eaten, the sugar may be absorbed so rapidly that the body is unable to convert it into glycogen fast enough to maintain the sugar concentration in the blood at a normal value. When the concentration in the blood exceeds 160 to 180 mg. per 100 ml., the sugar threshold is exceeded and sugar appears in the urine. The appearance of sugar in the urine under these conditions is known as **alimentary glycosuria** and is not serious. As soon as the excess sugar is eliminated, the individual returns to normal.

Some individuals have a very low sugar threshold, and sugar may occur in the urine when the blood sugar is normal. This condition is known as **renal diabetes.**

If a drug, phlorizin, is injected into an animal, sugar appears in the urine. This condition is known as **phlorizin diabetes** and has been used extensively in studying carbohydrate metabolism. Phlorizin acts by interfering with the phosphorylation of glucose, which slows up glucose metabolism in general. In a normal person glucose filters out of the blood in the kidney into the uriniferous tubules from which it is reabsorbed in the form of a phosphate. If phosphorylation does not occur, the glucose remains in the urine.

Another way of causing sugar to appear in the urine is to make what is known as a **diabetic puncture** on an animal by introducing a needle into the base of the brain. The stimulation of this part of the brain excites the nerves supplying the adrenal glands located above the kidneys. The adrenal glands liberate an internal secretion called **adrenaline,** which causes a rapid hydrolysis of glycogen by the liver. Sugar appears in the blood in such quantities that the sugar threshold is exceeded, and sugar appears in the urine.

During **pregnancy** glucose may appear in the urine even though the blood-sugar values do not increase appreciably. During lactation lactose may appear in the urine. This is often mistaken for glucose because it reduces Fehling's solution.

In certain **kidney diseases** glucose may appear in the urine with or without high blood-glucose values. When blood-glucose values remain normal, it is believed that the lining of the uriniferous tubules have degenerated to such an extent that reabsorption of glucose is interfered with.

Of especial interest is the appearance of sugar in the urine in the disease known as **pancreatic diabetes.** If a section of pancreas tissue, properly stained, is examined microscopically, certain groups of cells which stand out like little islands are observed. As these were first described by Langerhans, they have since been known as the **islands of Langerhans.** They produce not the pancreatic juice but a very important secretion which is absorbed directly into the blood. Such a secretion is known as an internal secretion or hormone. This particular hormone has been given the name **insulin.** It is now known that pancreatic diabetes is due to the absence or lack of sufficient quantity of insulin. If the pancreas is removed from an animal, diabetes develops immediately.

Insulin is a hormone that is essential for the storage of glycogen and for the utilization of sugar by the body. If it is lacking, sugar is not stored or utilized; and, when the concentration in the blood exceeds the sugar threshold, it is excreted in the urine. In diabetes, blood-sugar values may be very high: 300 to 500 mg. per 100 ml. is common, and as much as 1200 may be observed.

If diabetes simply meant an inability to utilize sugar, the situation would not be impossible, because the sufferer could still live on proteins and fats. However, since about 58 per cent of the amino acids in protein and the glycerol, or 10 per cent, of a fat may be converted into sugar in the body, these amounts of protein and fat also may not be utilized. Thus a diabetic would have to live on the fatty acids of the fat and about 40 per cent of the proteins in his food. The amino acids which do not change to sugar in the body are thought to be oxidized for the most part like fatty acids. Apparently there is a limit to the amount of fatty acid which the body is able to utilize for the production of energy. If this limit is passed, fats are incompletely oxidized, leaving acetoacetic acid [$CH_3—(C{=}O)—CH_2—COOH$], β-hydroxybutyric acid [$CH_3—CHOH—CH_2—COOH$], and acetone [$CH_3—(C{=}O)—CH_3$] as the end products instead of CO_2 and H_2O. Two of these substances are strong acids which tend to neutralize the alkalinity of the blood, thus producing a condition of **acidosis.** The severe acidosis which often accompanies diabetes is one of the dangerous features of the disease. From this discussion it can readily be seen that diabetes is a disease which not only interferes with carbohydrate metabolism but also upsets metabolism in general.

Diabetes is more common in people who are overweight than in those who are underweight. One of the first symptoms of the disease is a rapid loss in weight. Frequent urination is another symptom. The average volume of urine produced per day in a normal person is about 1 liter; in diabetics, 3 liters may be produced. Even though the volume of urine is large, the specific gravity remains high on account of the sugar present. The color of the urine is light.

If diabetes is recognized in its early stages, it can usually be controlled by diet. If the patient is overweight, it may mean that there is not sufficient insulin to supply the needs of so much tissue. A person may not have sufficient insulin to supply his needs if he weighs 250 lb., but he may have plenty after he has reduced his weight to 150 lb. In treating a diabetic patient by the diet method, carbohydrates and fats are limited in the diet. It is often possible to make the urine sugar-free by dieting, but the blood sugar may still be above normal and near the sugar threshold, that is, close to 160 mg. per 100 ml. The restricted diet should be continued until the blood-sugar value is normal. Usually it is necessary for the patient to continue on a low-sugar diet in order to maintain a normal blood-sugar value.

If the diabetic condition is so far advanced that it cannot be controlled by diet, it is possible to control it by means of insulin obtained from the pancreases of cattle. To be of value insulin must be injected into the muscle before eating. It is very important that the proper amount of insulin be injected each time. The quantity should be determined by a competent physician who studies each individual patient with the aid of blood-sugar determinations. Enough insulin is given to maintain the blood-sugar level at about 100 mg. per 100 ml. Each patient is, as it were, titrated with insulin to an end point which is a normal blood-sugar value. There is great danger in taking too much insulin; then the blood-sugar value becomes very low, and the patient may go into convulsions and die. An experienced insulin user can generally tell when he has taken too much insulin and can remedy the situation by eating sugar. Since insulin supplies the hormone which is lacking for the proper utilization of sugar, the insulin user does not have to be so careful to avoid carbohydrates in the diet as the diabetic who does not use insulin. He can to some extent choose the type of diet he desires and use a quantity of insulin sufficient to take care of these foods.

Mechanism of Insulin Action. Before glucose can be utilized by the body it must be converted into glucose-6-phosphate. This is accomplished, as mentioned above, by ATP in the presence of an enzyme called hexokinase. A hormone from the anterior pituitary gland inhibits the action of hexokinase. Insulin blocks the action of the pituitary hormone, thus allowing phosphorylation to occur and glucose metabolism to proceed normally.

It is apparent that insulin is not the only hormone associated with glucose metabolism. It has been pointed out that a diabetic puncture causes glycogenolysis owing to the action of adrenaline, a hormone of the adrenal medulla. Other hormones in the adrenal cortex and in the thyroid gland also influence glucose metabolism. If the diabetic condition is caused by the abnormal production of these other hormones rather than a decrease in insulin production the treatment of such a case with insulin is complicated. More will be said concerning these hormones in Chapter 20.

REVIEW QUESTIONS

1. Define metabolism.
2. What is meant by the terms endogenous and exogenous metabolism?
3. What is meant by the terms anabolism and catabolism?
4. Define glycogenesis, glycogenolysis, and glyconeogenesis.
5. What is meant by the sugar threshold?
6. What are the steps involved in the conversion of glucose into glycogen?
7. What are the steps involved in the conversion of liver glycogen into glucose?
8. Is muscle glycogen converted into glucose? Why?
9. What two stages are involved in the breakdown of glucose to carbon dioxide and water? Define glucolysis.
10. How does glucolysis in muscle differ from alcoholic fermentation by yeast?
11. Give the steps involved in the conversion of glucose-6-phosphate into lactic acid in muscle.
12. Give and explain the lactic acid cycle.
13. Give and explain the Krebs tricarboxylic acid cycle.
14. How may fat and amino acid metabolites fit into the Krebs cycle?
15. What is meant by low- and high-energy phosphate bonds?
16. Give examples of four types of high-energy phosphate bonds.
17. Explain the chemistry involved in muscular contraction.
18. What is meant by going into oxygen debt?
19. How may carbohydrates change to fats in the body?
20. How may carbohydrates change to amino acids in the body?
21. Discuss the origin of carbohydrates from proteins and from fats in the body.
22. What is meant by the G:N ratio? Of what value is it?
23. Under what conditions may sugar be found in the urine?
24. What is pancreatic diabetes?
25. What are the islands of Langerhans?
26. What is meant by acidosis? Under what conditions does it occur?
27. How is diabetes treated?
28. Give a theory explaining the mechanism of insulin action.
29. Are hormones other than insulin involved in glucose metabolism?

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13

Lipid Metabolism

During digestion, fats are hydrolyzed to glycerol and fatty acids. The glycerol, being soluble, is readily absorbed; the fatty acids are absorbed in combination with the bile salts. Most of the fat is absorbed through the lymphatic system. In the lymph and blood, fat is found, rather than the fragments of the fat molecules resulting from the digestion of fats. It is believed that the resynthesis of fat takes place in the intestinal lining. During the absorption of fat the concentration of both fat and phospholipid increases in the blood.

According to Frazer, fat is not completely hydrolyzed to glycerol and fatty acids in the intestine. That which is hydrolyzed is broken down into fatty acids and mono- and diglycerides. Much of the fat is absorbed as fat in the form of an emulsion. Some is absorbed as mono- and diglycerides combined with bile salts and fatty acids. Undigested fat and the mono- and diglyceride complex are absorbed into the lymph. Most of the fatty acids liberated during digestion are absorbed into the blood stream in combination with the bile salts.

Storage of Fat. From our discussion of the metabolism of carbohydrates it will be recalled that the first step in the oxidation of glucose is its combination with phosphoric acid. From the fact that phospholipids increase in the blood during the absorption of fats, it would appear that fats also combine with phosphoric acid as a first step in their metabolism. Phospholipids contain unsaturated fatty acid, which makes them more reactive than saturated fats. It is an interesting fact that the more active tissues of the body, such as those of the heart, contain the most unsaturated lipids. An important function of phospholipids appears to be that they aid in fatty acid transport in the body.

After entering the blood the fats and phospholipids are transported to all parts of the body and may be stored in various places as fat. Phospholipids are converted into fats before being stored.

The liver is a very important organ for the temporary storage of fat.

It is also an important site of fatty acid oxidation, as will be pointed out later. Much fat is stored under the skin in adipose tissue. This acts as an insulating material preventing the loss of heat from the body. A fat person is more comfortable in winter and more uncomfortable in summer than a thin person. Fat is also stored in the abdominal cavity, especially around the kidney; this is known as kidney fat. Another important place for fat storage is in intermuscular connective tissue. Such fat can be readily seen in a tender beef steak.

As a rule the composition of stored fat is rather uniform for a given species of animal. However, the composition may be varied by controlling the diet. If an animal is converting carbohydrate into fat, the fat synthesized will be of a type characteristic of the animal. On the other hand, if the stored fat is coming from fat in the food, it will tend to resemble the fat of the food. The fat of animals fed on feed rich in unsaturated oils will have a lower melting point than the fat from animals fed on carbohydrate or a saturated fat.

Isotopes and Fat-Metabolism Studies. One of the difficulties associated with the study of what happens to food molecules during metabolism is that after absorption they become mixed with similar molecules already present in the tissues and can no longer be identified as food molecules. If food molecules can be labeled so that they are identifiable at any stage of their metabolism, the problem is much simplified. Early attempts at labeling food molecules involved the substitution of a chlorine atom for a hydrogen atom. However, this method proved unsatisfactory because with the introduction of a chlorine atom the molecule was altered to such an extent that it no longer acted like a normal food molecule.

It is now possible to label food molecules by substituting isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, and sulfur for the normal elements originally present. The body is unable to distinguish between normal food molecules and those containing isotopes. Since we have a method for determining isotopic elements in a compound, we are able to follow a labeled food molecule through the various stages of its metabolism. (See p. 44.)

The most important early work on the use of isotopes in nutrition studies is that of Schoenheimer and Rittenberg, who fed rats fats and fatty acids containing heavy hydrogen tied to the carbon chain. In their first experiment they fed linseed oil which had been hydrogenated with heavy hydrogen. In 4 days the isotope content of the body fat showed that 44 per cent of the isotope fed had been deposited. This fact indicates that depot fats are not inert materials but are continually undergoing change.

In another experiment they fed, as the ethyl ester, palmitic acid in which 22 per cent of the hydrogen was of the heavy variety and, along with it, 6 per cent of butter. The rats were thus on a diet containing an ample supply of fatty acids, with one of them labeled with heavy hydrogen.

At the end of 8 days the body fats contained 44 per cent of the isotope fed, and, what is more interesting, the heavy hydrogen was now present in most of the fatty acids isolated from the mixture. This fact indicates that one fatty acid is constantly changing into other fatty acids during metabolism. The linoleic acid isolated was not isotopic, thus confirming the work of Burr and Burr, who showed that linoleic acid is essential in the diet.

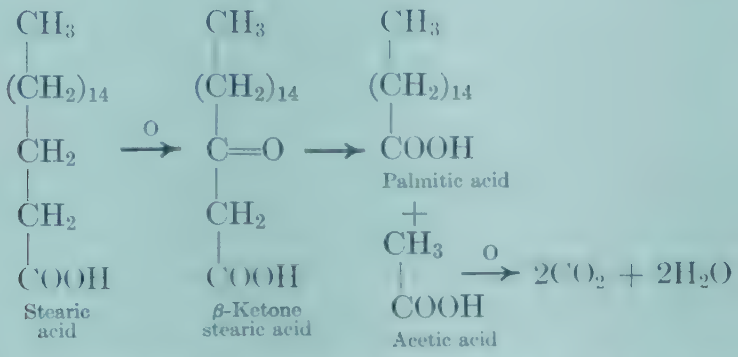
Another experiment was performed to demonstrate that fats may originate from carbohydrates in the body. This reaction involved a reduction of the sugar, in which the hydrogen for the reduction was derived from the water of the body tissues. Thus, if the water of the tissues contained heavy hydrogen, the resulting fats should contain it also. Mice injected with heavy water and given heavy water to drink soon showed heavy hydrogen in the fatty acids of their body fats. In this experiment no linoleic or linolenic acids containing heavy hydrogen were produced, indicating that these acids are not synthesized by the body. In general, it was found that about twice the quantity of saturated fatty acids was produced as of unsaturated. Oleic acid, containing heavy hydrogen, when oxidized at the double bond to form pelargonic and azelaic acids, showed heavy hydrogen in both these products. (See p. 108.) This indicates that the heavy hydrogen is distributed along the entire carbon chain in oleic acid and suggests that oleic acid is synthesized from small fragments of the sugar molecule.

Experiments indicate that the reactions just discussed take place mainly in the internal organs, such as the liver, rather than in the fat depots. In an experiment with a low-fat diet it was shown that half the fatty acids in the liver were synthesized in 1 day. Where depot fat was concerned, 1 week was required for the same result. These findings indicate that the main site of fatty acid synthesis and interconversion is the internal organs and that from here they are transported to the tissues and storage depots.

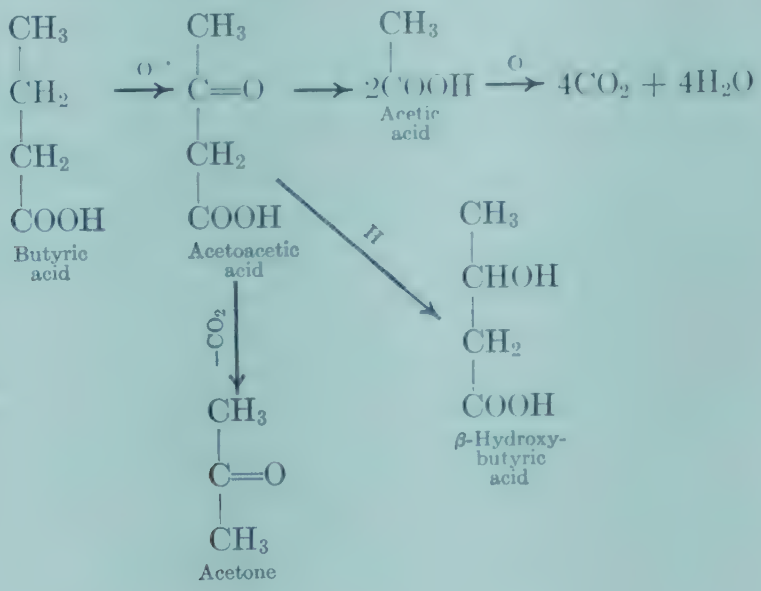
Oxidation of Fats. In the previous section we considered some of the general aspects of fat metabolism. It has been pointed out that fats are not stored in the fat depots as inert materials but are in dynamic equilibrium with the fat molecules of the food. There is a constant passage of fat molecules in and out of the fat depots, and one fatty acid is continually changing into another.

We next consider what happens to fats when they are oxidized in the body. Before fats are oxidized they are hydrolyzed to glycerol and fatty acids. The glycerol undoubtedly follows the route of carbohydrate metabolism after first being oxidized to glyceric aldehyde. Several theories have been advanced regarding the mechanism of fatty acid oxidation in the body.

Beta Oxidation. According to Knoop, long-chain fatty acids are oxidized at the β-carbon atom, forming a ketone acid which breaks down into acetic acid and an acid with two less carbon atoms than the original acid. The acetic acid is then oxidized to carbon dioxide and water.

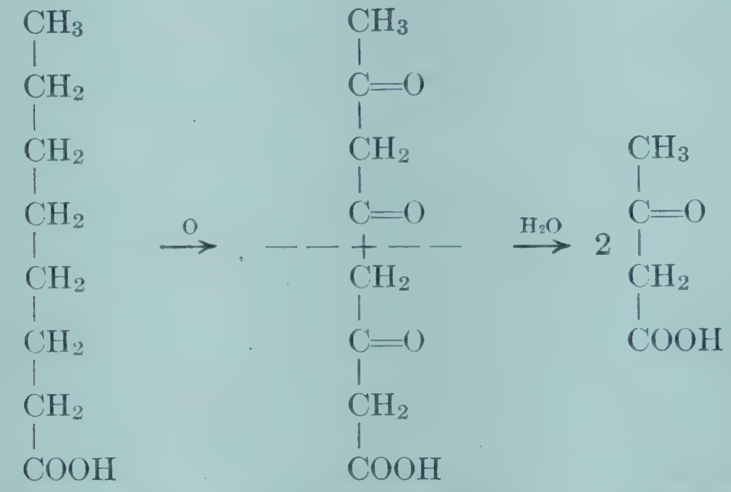

CH_3
$(CH_2)_{14}$
CH_2
CH_2
COOH
Stearic acid
O
CH_3
$(CH_2)_{14}$
C=O
CH_2
COOH
β-Ketone stearic acid
CH_3
$(CH_2)_{14}$
COOH
Palmitic acid
+
CH_3
COOH
Acetic acid
O
$2CO_2 + 2H_2O$

By repetition of this process butyric acid with four carbon atoms finally results. It is oxidized to acetoacetic acid, which may be broken down into acetic acid which is then oxidized to carbon dioxide and water. Some of the acetoacetic acid is reduced to β-hydroxybutyric acid and some is decarboxylated to form acetone. **Acetoacetic acid.**


CH_3
CH_2
CH_2
COOH
Butyric acid
O
CH_3
C=O
CH_2
COOH
Acetoacetic acid
CH_3
2COOH
Acetic acid
O
$4CO_2 + 4H_2O$
H
CH_3
CHOH
CH_2
COOH
β-Hydroxybutyric acid
$-CO_2$
CH_3
C=O
CH_3
Acetone

β-hydroxybutyric acid, and **acetone** are known as **acetone bodies** and are normally present in the blood in small amounts.

Multiple Alternate Oxidation. Jowett and Quastel have suggested a modification of the β-oxidation theory of fatty acid oxidation. According to this theory, starting with the β-carbon atom of a fatty acid, alternate carbon atoms are oxidized to ketones before the molecule breaks down, thus:



$$CH_3(CH_2)_6COOH \xrightarrow{O} CH_3{-}CO{-}CH_2{-}CO{-}{+}{-}CH_2{-}CO{-}CH_2{-}COOH \xrightarrow{H_2O} 2\,CH_3{-}CO{-}CH_2{-}COOH$$

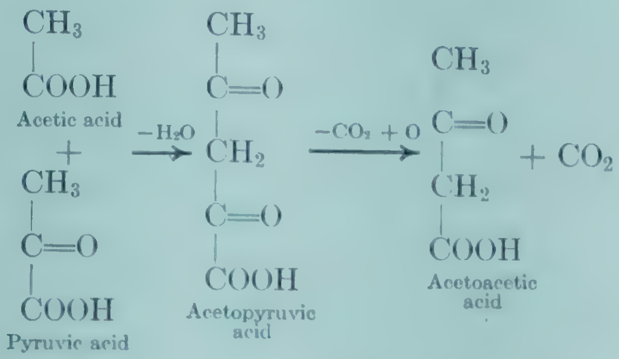
Caprylic acid — Oxidation product — Acetoacetic acid

According to the β-oxidation theory, each molecule of fatty acid, regardless of its size, should be able to give rise to one molecule of acetoacetic acid. Experiments have shown, however, that fatty acids containing eight or more carbon atoms give more acetoacetic acid than those with fewer carbon atoms. If fatty acids were oxidized according to the multiple alternate oxidation theory, this fact would be accounted for.

Beta-Oxidation-Condensation Theory. This is a modification of the β-oxidation theory and was suggested by MacKay and co-workers. According to this theory, molecules of acetic acid split from fatty acid molecules during β oxidation condense to form acetoacetic acid.

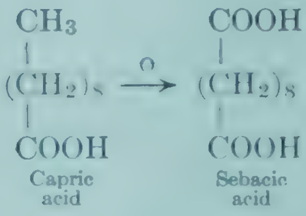
Evidence for this theory was obtained by incubating rat-liver slices with a fatty acid containing C^{13} in the carboxyl group. According to the β-oxidation theory, no C^{13} should appear in acetoacetic acid since it would be removed in the acetic acid formed in the first stage of the oxidation. According to the multiple alternate oxidation theory, the C^{13} should appear only in the carboxyl group of some of the acetoacetic acid formed. Actually it was found in both the carboxyl and carbonyl groups of some of the acetoacetic acid formed. This can be explained if two molecules of acetic acid containing C^{13} in the carboxyl group condensed to form acetoacetic acid.

Krebs-Johnston Theory. According to this theory, acetoacetic acid is obtained when acetic acid, resulting from β oxidation, condenses with pyruvic acid to form acetopyruvic acid. This on decarboxylation and oxidation gives acetoacetic acid.



$$CH_3COOH + CH_3COCOOH \xrightarrow{-H_2O} CH_3COCH_2COCOOH \xrightarrow{-CO_2\ +\ O} CH_3COCH_2COOH + CO_2$$

Omega Oxidation. It has been shown that, when certain artificial glycerides are fed, dicarboxylic acids are excreted in the urine. These acids contain the same number of carbon atoms as the fatty acid contained in the glyceride fed. This fact can be explained on the assumption that the end methyl group of the fatty acid has been oxidized to carboxyl, thus:



$$CH_3(CH_2)_8COOH \xrightarrow{O} COOH(CH_2)_8COOH$$

The carbon atom of the end methyl group is called the omega (ω)-carbon atom; hence this type of oxidation is called omega oxidation. Dicarboxylic acids may be further oxidized in the body by β oxidation at both ends of the molecule. The longer the carbon chain is, the more easily β oxidation proceeds. When acids with more than eleven carbon atoms are fed, they do not appear in the urine as dicarboxylic acids. Either ω oxidation does not occur with these acids, or the dicarboxylic acid formed has been further oxidized.

Just how important ω oxidation is in the oxidation of fatty acids is not known. It is possibly not of great importance, at least not so important as the other types of oxidation mentioned.

Stages in Fatty Acid Metabolism. The oxidation of fatty acids in the body takes place in two stages: (1) the oxidation to acetoacetic acid, and (2) the oxidation of acetoacetic acid to carbon dioxide and water. The first stage takes place in the liver and the second in the muscles and possibly in other tissues.

Ordinarily the muscles get their energy from the oxidation of glucose which is stored in large quantities in the liver as glycogen. Whenever the glycogen reserve in the liver becomes low, fatty acids are oxidized in the liver to acetoacetic acid. This passes into the blood and is carried to the muscles and other tissues. Here acetoacetic acid is hydrolyzed to acetic acid which enters the Krebs cycle of carbohydrate metabolism by combining with oxaloacetic acid to form citric acid. (See Fig. 21.) Thus the final stages of fatty acid metabolism are the same as those for carbohydrate metabolism.

Ketosis. We have just seen how acetoacetic acid may be oxidized in muscles for energy. However, there appears to be a limit to the amount of acetoacetic acid that the muscles are able to oxidize. Their total energy requirement cannot be obtained from this source alone. In starvation and in pancreatic diabetes when glycogen reserves are depleted the liver supplies so much acetoacetic acid to the muscles that they are unable to use all of it. That which is not used accumulates in the blood, together with β-hydroxybutyric acid and acetone. The two last-named compounds are derived from acetoacetic acid by reduction and decarboxylation, respectively. (See p. 257.) Acetoacetic acid and acetone are ketones, and their accumulation in the blood is called ketosis.

Ketosis is a dangerous condition because acetoacetic acid and β-hydroxybutyric acid reduce the alkalinity of the blood, producing a condition known as acidosis. If the alkalinity is reduced sufficiently, the person may go into coma or even die.

It is evident from what has been said that fats are **ketogenic** foods. Carbohydrates, which prevent the formation of acetone bodies, are said to be **antiketogenic** foods. The glycerol part of a fat which is oxidized in the body like a carbohydrate is an antiketogenic substance. The amino acids derived from proteins are of two types as far as the manner in which they are oxidized in the body is concerned. Some, like alanine, are oxidized like carbohydrates and are said to be antiketogenic. Others, like phenylalanine, form acetoacetic acid as an intermediate product of metabolism and thus oxidize like fats and are ketogenic in nature.

If too much fat is included in the diet, there is danger of ketosis even in a normal individual. In order that ketosis be prevented, it is essential that there be sufficient antiketogenic substance in the diet to balance the ketogenic. The ratio of ketogenic to antiketogenic substance in the diet is known as the **ketogenic : antiketogenic ratio.**

Studies of this ratio in man indicate that the amount of fat may equal twice the weight of carbohydrate plus one-half the weight of protein in the diet without ketosis developing. Thus a person eating 100 grams of carbohydrate and 50 grams of protein should be able to eat 225 grams of fat without developing ketosis. An understanding of the ketogenic : antiketogenic balance is important in dietetics, especially in the medical field. Under certain conditions it may be desirable for a patient to have a ketogenic diet. This has been found to be true in epilepsy.

Fat as a Source of Energy for Work. It is known that during muscular work fats are oxidized. Evidence in support of this view has been obtained from respiratory-quotient studies and from the fact that work causes a rise in the concentration of lipids in the blood. We have just learned that when fatty acids are oxidized they form acetoacetic acid and that this hydrolyzes to form acetic acid which enters the Krebs cycle and is oxidized to carbon dioxide and water. The energy released is used to produce high-energy phosphate bonds, which are the immediate source of energy for muscular contraction. It is reasonable to assume that the energy derived from the oxidation of any foodstuff may be utilized for muscular contraction.

Origin of Fats from Carbohydrates and Proteins. Not all the fat in the body comes from the fat of the food. Carbohydrate is a very important source of body fat, as was explained in Chapter 12. As mentioned earlier in this chapter, Schoenheimer demonstrated the conversion of carbohydrates into fats by the use of isotopes. The origin of fat from protein is very difficult to demonstrate because proteins stimulate metabolism to such an extent that food materials are oxidized rather than stored as fat. However, since it has been shown that in diabetes proteins may change to sugar, which is eliminated in the urine, it is highly probable that under normal conditions also proteins may change to sugar during metabolism. Since sugar may change to fat, it is very likely that indirectly fat may come from protein.

Longenecker has demonstrated experimentally that protein is converted into fat in rats. He fed rats that had been starved to reduce their fat reserves a diet consisting of casein, yeast, and mineral salts and found that they regained their weight, and an analysis of their tissues showed that much of the new tissue was fat. Since the diet was largely protein and contained little carbohydrate or fat, the deposited fat must have come from protein.

Essential Fatty Acids. We have previously pointed out that certain unsaturated fatty acids are indispensable in the diet and that the body is unable to synthesize these acids. In 1929 Burr and Burr described an experiment in which they showed that linoleic, linolenic, or arachidonic acids must be supplied in the diet of rats. If rats are fed on a fat-free diet, their growth is retarded, their skin becomes scaly, their tails become deformed, and they are unable to reproduce. Finally, kidney lesions develop, the urine becomes bloody, and death follows. If these rats are fed any one of the above-mentioned unsaturated fatty acids, the condition clears up. Linoleic acid is the most effective in bringing about a cure of the disease and may be considered the essential unsaturated fatty acid, the other acids being able to replace it if necessary.

The importance of the essential fatty acids in human nutrition has been demonstrated by Hansen. Certain types of eczema in infants have been cured by feeding fats containing the essential fatty acids.

Obesity. Obesity is a condition in which too much fat is deposited in the body, resulting in overweight. Undoubtedly obesity is sometimes due to an abnormal condition of certain endocrine organs, but usually it is simply a result of eating more food than the body requires. It is common knowledge that a thin person may eat much more than a fat person but be unable to put on weight, whereas a fat person may appear to eat very little and still not get thin. As a rule thin people are more active than fat people, a fact which may account for the apparent discrepancy. According to the law of conservation of energy, it is apparent that a thin person eating more than he needs should put on weight, and an obese person eating less than is required for metabolic purposes should get thin.

One factor which is often overlooked by a person who is attempting to get thin by dieting is that body tissues are composed largely of water and that, unless a dietary régime is continued for a long period of time, there may simply be a replacement of fat by water with an actual increase in weight over a short period of time. The author knows of one person who attempted to lose weight by eliminating breakfast and lunch and eating only a moderate amount at dinner each day. In order to satisfy her hunger, she drank quantities of water during the day. At the end of a month she had lost 1 pound and gave up the attempt in disgust. Undoubtedly water had replaced fat in this person's tissues.

The relation of water to body weight should not be overlooked by persons who are attempting to gain weight. Physicians often recommend an increase in the consumption of water as a remedy for emaciation. As a rule it will be found that thin people habitually drink less water than obese.

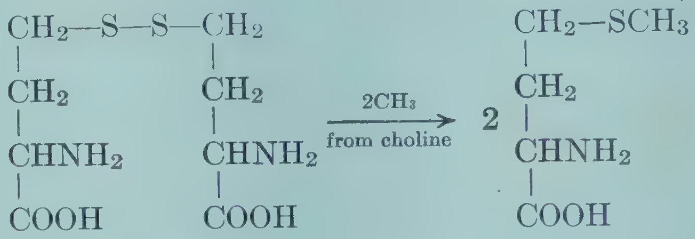
Perhaps the most important factor in the control of body weight is appetite, which is nature's way of controlling the body's intake of energy in the form of food. It is remarkable how, without apparent effort, the average person consumes just the right amount of food for his needs and maintains a fairly constant weight over long periods of time. Perhaps the real cause of obesity or emaciation is that in the obese person appetite is slightly greater than it should be, whereas in the thin one it is less. The average person, in the long run, will eat what his appetite dictates. In an adult this quantity should be only enough to maintain normal weight. If appetite could be controlled in a simple manner, it should not be difficult to control body weight.

Choline and Fat Metabolism. In the more active tissues of the body lecithin and other phospholipids, rather than neutral fat, are found. In fact, lecithin appears to be a constituent of every living cell. Lecithin differs from a fat in that phosphoric acid and choline replace one of the fatty acids, and one of the fatty acids is unsaturated. The phosphoric acid-choline complex and the unsaturated fatty acid make the lecithin molecule more diffusible and oxidizable than a neutral fat molecule. If there is insufficient choline in the diet, an animal fed a diet rich in fat deposits large quantities of fat in the liver. This development of fatty livers may be prevented by the inclusion of lecithin or choline in the diet.

The relation of choline deficiency to the formation of fatty livers has been explained in the following manner. If the supply of choline is low, the formation of lecithin in the liver is interfered with. Since lipids are transported in the body mainly as lecithin, any interference with the formation of lecithin will interfere with lipid transfer. Thus lipids reaching the liver which cannot be changed to lecithin will be deposited unchanged in the liver.

Substances such as lecithin and choline which prevent the formation of fatty livers are said to be **lipotropic.** There are other compounds besides lecithin and choline which are lipotropic. Among them may be mentioned betaine (see p. 126) and the amino acid methionine. It is thought that these compounds are effective because they contain labile methyl groups which may be used to synthesize choline in the body. It will be recalled that choline contains three methyl groups and that its chemical name is hydroxyethyltrimethylammonium hydroxide. Proteins, such as casein, which contain a high percentage of methionine are lipotropic.

Transmethylation. Besides being important in fat metabolism, choline plays a significant role in other biological reactions involving the transfer of methyl groups from it to other compounds. This transfer of methyl groups is called transmethylation. Du Vigneaud has shown that rats fed a diet containing no methionine, an essential amino acid, do not grow; but, when homocystine and choline are supplied, growth is normal. This fact indicates that homocystine is converted to methionine by a process of transmethylation, the methyl group coming from choline.



$$\begin{array}{ll} CH_2\text{—}S\text{—}S\text{—} & CH_2 \\ | & | \\ CH_2 & CH_2 \\ | & | \\ CHNH_2 & CHNH_2 \\ | & | \\ COOH & COOH \end{array} \xrightarrow[\text{from choline}]{2CH_3} 2 \begin{array}{l} CH_2\text{—}SCH_3 \\ | \\ CH_2 \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$

Homocystine Methionine

As indicated in the previous section, methionine and betaine may act as transmethylating agents by supplying methyl groups for the synthesis of choline.

Another example of transmethylation is the formation of creatine from glycocyamine and methionine. (See p. 275.)

It is interesting to note that not all compounds containing methyl groups can serve as methylating agents. For example, creatine cannot serve in this capacity. The methyl group of creatine is not labile.

Transmethylation is a significant biological reaction because some very important compounds such as choline, methionine, and creatine may be synthesized in the body by this means. Methyl groups appear to be synthesized with difficulty by the animal body.

Metabolism of Cholesterol. Cholesterol is found in the unsaponifiable fraction of all animal fats. It is absorbed into the lymph as much of the fat is absorbed. Bile is necessary for cholesterol absorption. In the blood, cholesterol is found free and in combination with fatty acids as esters. The large amount of cholesterol esters in the blood suggests that an important function of cholesterol may be to transport fatty acids in the body.

It has been shown by the use of isotopes that cholesterol may be converted into bile salts in the body. It would appear that cholesterol may also be the parent substance of the sex and adrenal cortical hormones, which are sterols or sterol derivatives.

Although cholesterol is an essential compound for life, it is not necessary to supply it in the diet. It can be synthesized in the body. Bloch and Rittenberg fed rats sodium acetate containing heavy hydrogen and later found the heavy hydrogen in cholesterol obtained from the rats. Thus cholesterol may be synthesized from such a simple compound as acetic acid, an important metabolic intermediate.

Any excess of cholesterol in the blood is excreted by the intestines or by the liver in the bile. Cholesterol in the bile may precipitate in the gall bladder, forming gallstones.

In **atherosclerosis,** a disease usually associated with high blood-cholesterol values, cholesterol together with calcium salts deposits in the arteries resulting in "hardening of the arteries." Formerly it was thought that persons with atherosclerosis should avoid eating animal fats which contain cholesterol. It now appears that the amount of cholesterol in the diet has little effect on the cholesterol content of the blood, and diets low in animal fats are of little value in this disease. Apparently the high cholesterol content of the blood is produced through synthesis.

REVIEW QUESTIONS

1. How are fats absorbed from the digestive tract?
2. Name several places where fats are stored in the body.
3. In what form are fats stored in the body?
4. What are isotopes, and how are they used in metabolism studies?
5. Outline briefly Schoenheimer and Rittenberg's work on the use of isotopes in fat-metabolism studies.
6. Name and describe five theories of how fatty acids are oxidized in the body.
7. Name the acetone bodies, and indicate how each may come from butyric acid.
8. What evidence supports the multiple alternate, β-oxidation-condensation, and ω-oxidation theories of fatty acid oxidation?
9. Name the two stages in the metabolism of fatty acids. Where does each stage occur? What chemical changes take place during each stage?
10. What is ketosis and under what conditions does it occur?
11. What is meant by ketogenic and antiketogenic foods?
12. How much fat may be included in the diet in relation to carbohydrate and protein intake without ketosis developing?
13. Are fats used as a source of energy for work?
14. Can body fats originate from carbohydrates and proteins in the food?
15. What fatty acids are indispensable in the diet? What happens to rats and human infants if these acids are lacking in the diet?
16. Discuss obesity.
17. Discuss the relation of choline to the formation of fatty livers.
18. Name four lipotropic substances.
19. What is meant by transmethylation? Give an example in which choline acts as a methylating agent.
20. Name three functions of cholesterol in the body.
21. Can the body synthesize cholesterol?

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14

Protein Metabolism

During digestion, proteins are broken down into their constituent amino acids, which are absorbed directly into the blood stream. The amino acids are carried by the blood to the liver and finally to all the tissues of the body. The proteins differ from the carbohydrates and fats in that their prime function is the building of body tissue. The amino acids have been called the building stones out of which body tissue is constructed. After the amino acids are absorbed, they are presented to the tissues, and those which are needed for building new tissue or replacing worn-out tissue become a part of the living tissue. Simultaneously with this synthesis there is a constant breakdown of body proteins, and the resulting amino acids become intimately mixed with those derived from the food. So it is impossible to say that any given amino acid is used for tissue building or that it is not. It is possible that every amino acid in the food during its passage through the body may at some time be a part of the living tissue.

Besides these reactions involved in the synthesis and degradation of tissue, the amino acids undergo many other reactions in the course of their metabolism. The amino groups of one amino acid may replace the amino groups of another, or new amino acids may be formed by the transfer of amino groups to compounds derived from carbohydrate or fat metabolism. Eventually amino acids are oxidized in the body to form carbon dioxide, water, and ammonia. In man most of the ammonia is converted in the liver to urea, in which form it is eliminated in the urine. It is thus evident that the metabolism of proteins is a complicated affair.

Nitrogenous Equilibrium. Unlike carbohydrates and fats, proteins are stored in the body in only limited amounts. Within a very few hours after a protein meal all the nitrogen is eliminated, mainly in the urine. An adult who receives an adequate amount of protein in his diet and who is neither losing nor gaining in weight will excrete in the urine, feces, and perspiration an amount of nitrogen equal to that in the food. Such a person is said to be in a state of **nitrogenous equilibrium.** If the amount of nitrogen in the excreta is greater than that in the food, the person is said to have a **negative nitrogen balance.** Such a condition prevails during starvation, during wasting diseases, and if the protein eaten does not contain the correct mixture of amino acids. In growing children less nitrogen is eliminated in the excreta than is present in the food, showing that there is a retention of nitrogen by the body. Such an individual is said to have a **positive nitrogen balance.**

Even if a person receives no nitrogen in his food, nitrogen is excreted. This nitrogen comes from the breakdown of tissue. If a person goes without food, the nitrogen excreted rapidly approaches a minimum and stays there as long as the fat and glycogen reserves hold out. Finally body proteins are called upon for supplying energy, and the nitrogen excreted increases rapidly. When the tissues have supplied all the protein they can spare for energy production, the nitrogen excreted decreases until finally death occurs.

An important question for nutrition is to know the minimum amount of protein required to maintain nitrogenous equilibrium. One investigator has reported a person in whom nitrogenous equilibrium was maintained on as little as 15 grams of protein per day. To accomplish this, the protein eaten must have contained an excellent assortment of amino acids. Ordinarily we eat much more protein. As a rule a 24-hour sample of urine contains about 15 grams of nitrogen, which corresponds to about 94 grams of protein. The Committee on Food and Nutrition of the National Research Council recommends a daily allowance of 70 grams of protein in the diet of a normal adult.

Protein Storage. In the previous section it was stated that the storage of protein in the body is limited. Carbohydrates are stored as glycogen in the liver and muscles. An increase in the amount of fat in the body is easily demonstrated. But, since most of the body tissues are composed of protein, it is difficult to decide whether an increase in the weight of these tissues should be considered as a storage of protein or as growth.

If an animal is fed a high-protein diet, the amount of protein in the liver, kidneys, and intestinal tissue increases rapidly. If protein is then eliminated from the diet, these tissues lose protein quickly. This indicates that protein has been stored in these tissues in a form that is quickly available for use when needed.

Another evidence of protein storage in the liver and other tissues is provided by **plasmapheresis** experiments. In plasmapheresis blood is withdrawn from an animal and the cells are separated from the plasma. The cells are then suspended in salt solution and reinjected into the blood stream of the animal. Thus the blood loses plasma with its proteins. After such an experiment the amount of protein in the plasma rapidly increases. It is believed that the protein for the regeneration of plasma proteins comes from that stored in the liver and other tissues.

Thus it would appear that there is a storage of protein in the body, especially in the liver, kidney, and intestine. It is believed that the protein is stored as protein and not as amino acids.

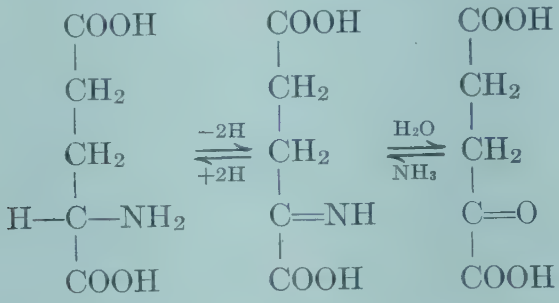
Metabolism of Tissue Proteins. The tissues of the body are composed mainly of protein. This tissue protein originates largely from the amino acids resulting from the digestion of food proteins. The amount of protein in the body is fairly constant. If more protein is eaten in a day than the body requires, the nitrogen of the protein is soon found in the urine in the form of urea or other end products of protein metabolism. There is, however, a constant need for proteins in the diet to meet the requirements for building new tissue and for maintaining that tissue which is already present.

Our knowledge of the chemistry involved in the synthesis and degradation of body-tissue proteins has been advanced considerably through the work of Schoenheimer and others with isotopes. By introducing heavy hydrogen into the carbon chain and heavy nitrogen (N^{15}) into the amino group of amino acids, it has been possible to follow such amino acids through their various phases of metabolism. The heavy hydrogen acts as a marker for the carbon chains, and the heavy nitrogen for the amino group of the amino acids.

When isotopic leucine was added to the normal diet of rats in nitrogenous equilibrium, at the end of 3 days it was found that 56.5 per cent of the isotopic nitrogen was deposited in the tissues. On further analysis it was shown that the heavy nitrogen appeared not only in the leucine of the tissues but also in most of the other amino acids isolated. Lysine was an exception, there being no heavy nitrogen in the lysine isolated. Among the other amino acids studied, glutamic acid showed the largest amount of heavy nitrogen, excepting leucine. The leucine which was isolated contained a high percentage of heavy hydrogen, an indication that the leucine fed was utilized, at least in part, unchanged.

This experiment indicates that amino acids in the tissues are being rapidly exchanged for amino acids in the food, even though the animal is in a state of nitrogenous equilibrium. It also shows that the amino group of one amino acid may be used for the synthesis of another amino acid. The high concentration of heavy nitrogen in glutamic acid indicates that this amino acid has some special function in protein metabolism.

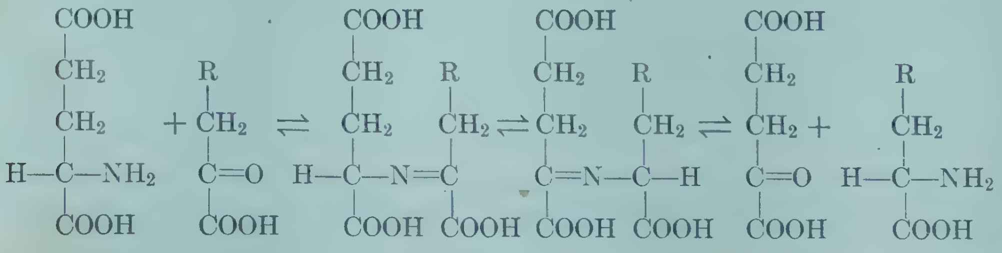
Transamination. The mechanism by which the amino group of one amino acid is transferred to another has been investigated by means of isotopes. A simple explanation is that an amino acid may undergo dehydrogenation and hydrolysis to form a ketone acid and ammonia. The ammonia formed may react with the same or some other ketone acid to form the original or some new amino acid.



$$\begin{array}{c}\mathrm{COOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{H{-}C{-}NH_2}\\ |\\ \mathrm{COOH}\end{array} \underset{+2H}{\overset{-2H}{\rightleftharpoons}} \begin{array}{c}\mathrm{COOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{C{=}NH}\\ |\\ \mathrm{COOH}\end{array} \underset{NH_3}{\overset{H_2O}{\rightleftharpoons}} \begin{array}{c}\mathrm{COOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{C{=}O}\\ |\\ \mathrm{COOH}\end{array}$$

If this reaction were taking place in body tissue, it should be possible to introduce heavy nitrogen into amino acids by feeding an animal ammonia containing heavy nitrogen. Such an experiment was performed by adding to the diet isotopic ammonium citrate. After 9 days the heavy nitrogen was found widely distributed among the amino acids of the body protein. Here again glutamic acid contained more heavy nitrogen than any other amino acid, thus emphasizing the importance of this amino acid.

Although the foregoing scheme accounts very nicely for the transfer of amino groups from one amino acid to another, it does not exclude other possibilities. Amino acids will react with ketone acids to form substituted imino acids, which on hydrolysis may form a new amino acid. In this reaction no ammonia is involved. The transfer of an amino group from an amino acid to a ketone acid in this manner is called **transamination.**

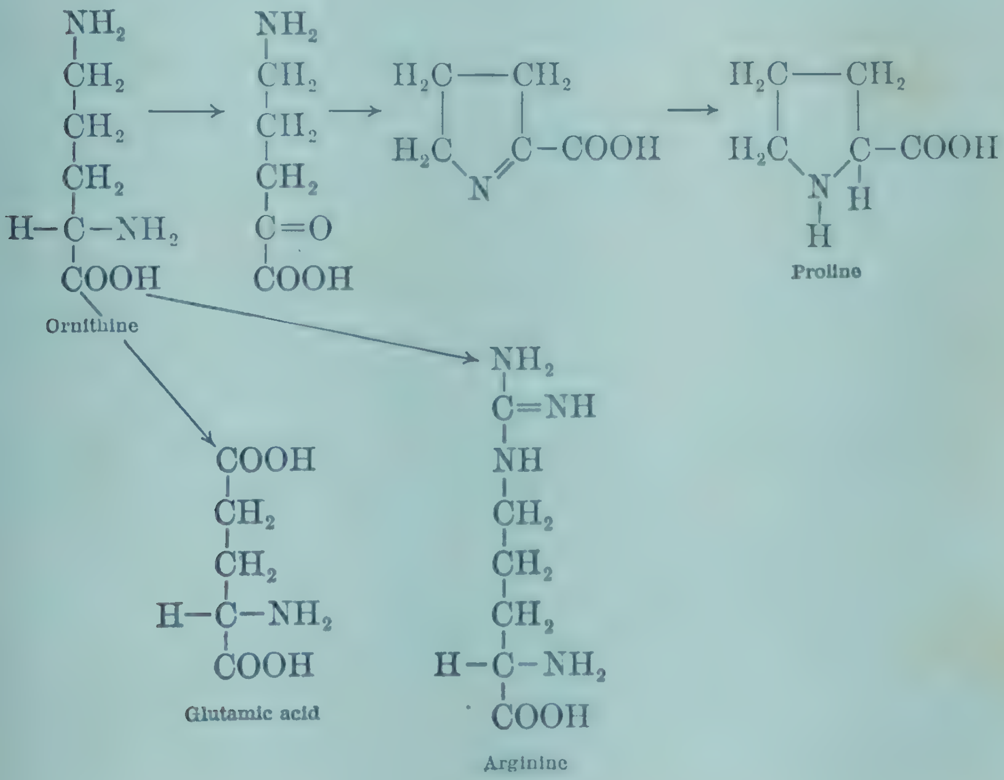


$$\begin{array}{c}\mathrm{COOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{H{-}C{-}NH_2}\\ |\\ \mathrm{COOH}\end{array} + \begin{array}{c}\mathrm{R}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{C{=}O}\\ |\\ \mathrm{COOH}\end{array} \rightleftharpoons \begin{array}{cc}\mathrm{COOH} & \\ | & \mathrm{R}\\ \mathrm{CH_2} & |\\ | & \mathrm{CH_2}\\ \mathrm{H{-}C{-}N{=}} & \mathrm{C}\\ | & |\\ \mathrm{COOH} & \mathrm{COOH}\end{array} \rightleftharpoons \begin{array}{cc}\mathrm{COOH} & \\ | & \mathrm{R}\\ \mathrm{CH_2} & |\\ | & \mathrm{CH_2}\\ \mathrm{C{=}N{-}} & \mathrm{C{-}H}\\ | & |\\ \mathrm{COOH} & \mathrm{COOH}\end{array} \rightleftharpoons \begin{array}{c}\mathrm{COOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{C{=}O}\\ |\\ \mathrm{COOH}\end{array} + \begin{array}{c}\mathrm{R}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{H{-}C{-}NH_2}\\ |\\ \mathrm{COOH}\end{array}$$

As evidence that transamination really occurs in tissues, it may be stated that a transaminating enzyme has been prepared from muscle tissue. This enzyme works only when glutamic or aspartic acid or the ketone derivatives of these acids are present. Thus a reason is suggested for the findings reported above, in which glutamic acid appears to be so active in the exchange of amino groups. It has been suggested that in the exchange of amino groups in metabolism the ammonia derived from the deamination of amino acids is first converted into glutamic acid, which in turn transfers its amino group to ketone acids by the process of transamination.

It should be noted at this point that the ketone acid derived from glutamic acid is α-ketoglutaric acid. It will be recalled that α-ketoglutaric acid is one of the intermediates in carbohydrate metabolism. This fact suggests a route by which carbohydrates may be converted in the body to amino acids and protein. α-Ketoglutaric acid reacts with ammonia to form glutamic acid, and then by transamination other amino acids may be formed.

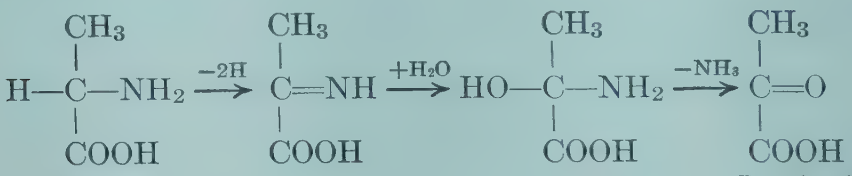
That the carbon chain in one amino acid may be converted into another amino acid has been demonstrated by feeding rats ornithine with heavy hydrogen tied to the carbon chain. A few days later proline, glutamic acid, and arginine, which contained heavy hydrogen in the ring or carbon chain, were isolated from the rat tissues. The following diagram indicates the chemical relationship of ornithine to proline, glutamic acid, and arginine.



When phenylalanine containing heavy hydrogen in the ring was fed to rats, tyrosine containing heavy hydrogen in the ring was isolated from the body proteins. Thus tyrosine may be derived from phenylalanine in the diet.

Oxidation of Amino Acids. So far our discussion of protein metabolism has dealt mainly with the relation of amino acids to tissue building. A very important phase of protein metabolism deals with the final oxidation of amino acids and their conversion into metabolic end products which are eliminated from the body. In studying protein metabolism we find that the urine contains most of the end products which have nitrogen. Among them the most important are urea, ammonia, creatinine, and uric acid.

Formation of Urea. The first thing that happens to an amino acid in its catabolism is the removal of its amino group with the formation of a ketone acid and ammonia. The reaction possibly takes place in several stages. There is first a removal of hydrogen by a dehydrogenase enzyme. This step is followed by the addition of water and the removal of ammonia, thus:

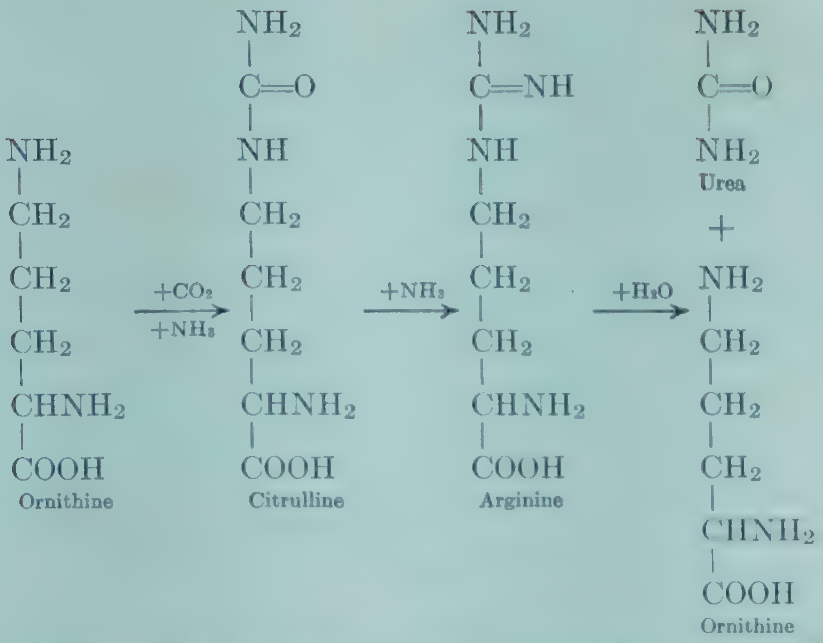


$$\begin{array}{c}CH_3\\|\\H-C-NH_2\\|\\COOH\end{array} \xrightarrow{-2H} \begin{array}{c}CH_3\\|\\C=NH\\|\\COOH\end{array} \xrightarrow{+H_2O} \begin{array}{c}CH_3\\|\\HO-C-NH_2\\|\\COOH\end{array} \xrightarrow{-NH_3} \begin{array}{c}CH_3\\|\\C=O\\|\\COOH\end{array}$$

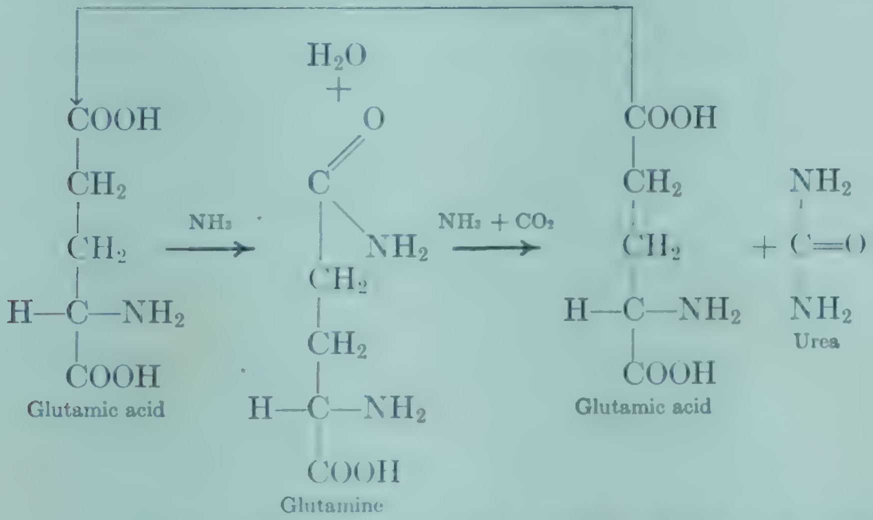
Alanine Pyruvic acid

Deaminization of amino acids is thought to occur in many of the body tissues, but principally in the liver and kidney. According to Krebs and Henseleit, the ammonia formed in the process is converted into urea in the liver by a rather complicated process involving the amino acid **ornithine** and the enzyme **arginase.** Ornithine is a diamino acid derived from arginine by removing the amidine group. It reacts with carbon dioxide and ammonia to form **citrulline.** With another molecule of ammonia citrulline forms **arginine.** In the presence of the enzyme **arginase,** which is found in the liver, arginine is hydrolyzed to form one molecule of urea and one of ornithine. Ornithine is thus used over and over again. The reactions involved may be represented as shown at the top of p. 273.

According to Leuthardt, urea is formed from glutamine or asparagine reacting with ammonia and carbon dioxide. Glutamine and asparagine are the amides of glutamic and aspartic acid, respectively, and may be looked upon as carriers of ammonia. Thus glutamine and asparagine, rather than ammonium salts, are found in the blood.


+CO₂
+NH₃
+NH₃
+H₂O
Ornithine
Citrulline
Arginine
Urea
Ornithine

Using glutamine as an example the synthesis of urea may be represented as follows:


NH₃
NH₃ + CO₂
H₂O
Glutamic acid
Glutamine
Glutamic acid
Urea

Urea, as it is formed, is carried to the kidneys, where it is eliminated in the urine. In a normal person from 80 to 90 per cent of the nitrogen in the urine is in the form of urea. The quantity of urea in the urine varies from day to day, depending upon the protein intake.

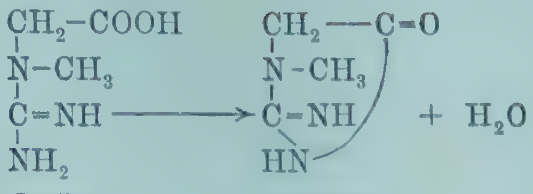
The ketone acids resulting from the deaminization of amino acids are eventually oxidized to carbon dioxide and water for energy production. The ketone acid formed when alanine is deaminized is pyruvic acid, which will be recognized as an intermediate product in the metabolism of glucose. Thus alanine on oxidation goes the route of carbohydrate metabolism. Since most reactions are reversible, the pyruvic acid formed from alanine, instead of being oxidized directly, may be converted into glucose. Such a conversion of amino acids undoubtedly takes place in diabetes, where it has been demonstrated that proteins may give rise to sugar in the urine. Since we know that glucose may be converted into fat in the animal body, it is undoubtedly true that proteins may give rise to fat in the body.

Formation of Ammonia. About 4 per cent of the nitrogen in normal urine is in the form of ammonium salts. In view of the fact that there is a very low concentration of ammonia in the blood, it becomes interesting to contemplate the origin of the large quantities of ammonia in the urine. At one time it was thought that the kidney could convert urea back to ammonia. Now it is believed that urinary ammonia is derived from the deaminization of amino acids in the kidney. According to Van Slyke, the main precursor of urinary ammonia is glutamine, the amide of glutamic acid.

The source of urinary ammonia has been investigated by means of isotopes. If an animal is fed urea containing isotopic nitrogen, the isotopic nitrogen is found to be still in the form of urea in the urine. Thus urea is not converted into ammonia in the body. If an animal is fed ammonium salts containing isotopic nitrogen, the isotopic nitrogen appears in the urine as urea. The ammonia is converted into urea in the liver. However, if an animal is fed amino acids containing isotopic nitrogen, the ammonium salts in the urine contain a large percentage of isotopic nitrogen. Thus it appears that urinary ammonia comes from the deaminization of amino acids.

Creatine and Creatinine. These two nitrogenous compounds, found widely distributed in the body, are products of protein metabolism. Creatine is found especially in the muscles in combination with phosphoric acid as phosphocreatine and is intimately associated with the chemical changes taking place during muscular contraction. (See p. 245.)

Creatine is not found to any appreciable extent in the urine. There is some in the urine of women and children, but in men there is normally none. Creatinine, on the other hand, is present in tissues in only small amounts but is always found in the urine in rather large amounts. The amount of creatinine excreted per day is quite constant for a given individual and is independent of the protein intake. It apparently is derived from the creatine of the tissues and may be considered a waste product. Creatine and creatinine are closely related chemically. Creatine is methylguanidine acetic acid, and creatinine is the anhydride of creatine.

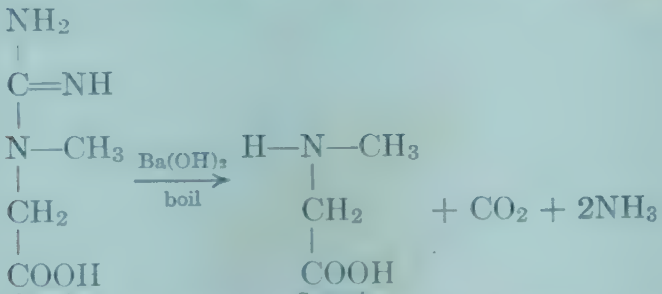


Creatine Creatinine

That creatine may be converted to creatinine in the body has been shown by feeding an animal isotopic creatine and recovering isotopic creatinine in the urine. That the process is irreversible is shown by the fact that when isotopic creatinine is fed it may be recovered in the urine, but no isotopic creatine appears in the tissues.

Origin of Creatine. There has been much speculation as to the origin of creatine. As a result of isotopic studies it is now possible to state quite definitely from what precursors creatine and creatinine originate in the body. Creatine contains three nitrogen atoms, and it is obvious that they must come from amino acids. When various amino acids containing heavy nitrogen are fed, it is found that only **arginine** and **glycine** contribute their nitrogen to creatine.

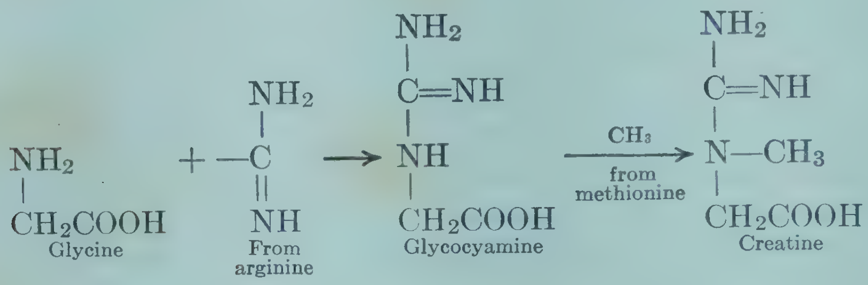
When creatine is boiled with alkali, it is decomposed as follows:



Creatine Sarcosine

The ammonia given off is derived from the amidine group; the other nitrogen remains in the sarcosine molecule. When arginine containing isotopic nitrogen in the amidine group is fed, the isotope appears in the ammonia fraction. Thus arginine furnishes the amidine group of creatine. When glycine containing isotopic nitrogen is fed, the isotype appears in the sarcosine fraction. Thus the nitrogen of the sarcosine part of the creatine molecule must come from glycine.

The combination of the amidine group of arginine with glycine gives glycocyamine, which must be methylated to form creatine. Glycocyamine is converted into creatine by liver tissue, but the process is very slow; when **methionine** is present, however, the conversion takes place rapidly. Thus methionine appears to be the methylating agent responsible for the reaction. As final proof, du Vigneaud fed rats methionine with heavy hydrogen in the methyl group and isolated creatine with the isotope in the methyl group. The biological synthesis of creatine from glycine, arginine, and methionine may be expressed diagrammatically as follows:



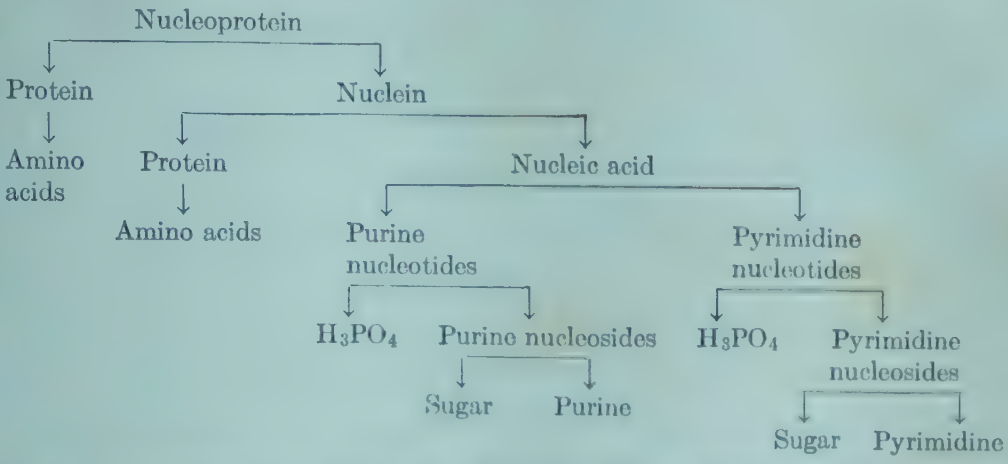
Besides methionine, choline and betaine may serve as methyl donors in the above reaction. It should be pointed out that creatine, although it contains a methyl group, cannot act as a methyl donor in transmethylation reactions.

Nucleoprotein Metabolism. It will be recalled from the discussion of the chemistry of nucleoproteins that they are conjugated proteins composed of protein and nucleic acid. It will also be recalled that nucleic acid is a polynucleotide, each nucleotide being composed of phosphoric acid, a sugar, and either a purine (adenine or guanine) or a pyrimidine (cytosine, uracil, or thymine).

When nucleoprotein is eaten, enzymes in the intestine hydrolyze it into protein and nuclein. Nuclein is further hydrolyzed into protein and nucleic acid. The proteins resulting from the hydrolysis of nucleoprotein and nuclein are further hydrolyzed by the proteolytic enzymes of the intestine to amino acids, as is any protein eaten. The nucleic acid is first hydrolyzed by an enzyme called polynucleotidase to form nucleotides. The nucleotides are hydrolyzed by an enzyme called phosphatase to form nucleosides. In this reaction phosphoric acid is removed from each nucleotide. Finally an enzyme called nucleosidase splits the nucleosides into sugar and purine.

There is said to be no enzyme capable of hydrolyzing pyrimidine nucleosides in the intestine. It would appear, however, that such a reaction should occur eventually in the body during or after absorption. It has been suggested that it may occur in the intestinal lining.

The diagram on p. 277 shows the steps in the digestion of nucleoprotein. Thus nucleoprotein is hydrolyzed during digestion into the simple units of which it is composed. The amino acids are utilized, as is any amino acid derived from other proteins. The sugars which are pentoses may be used for the resynthesis of nucleic acid. The phosphoric acid may be used for the resynthesis of nucleic acid and for the syn-



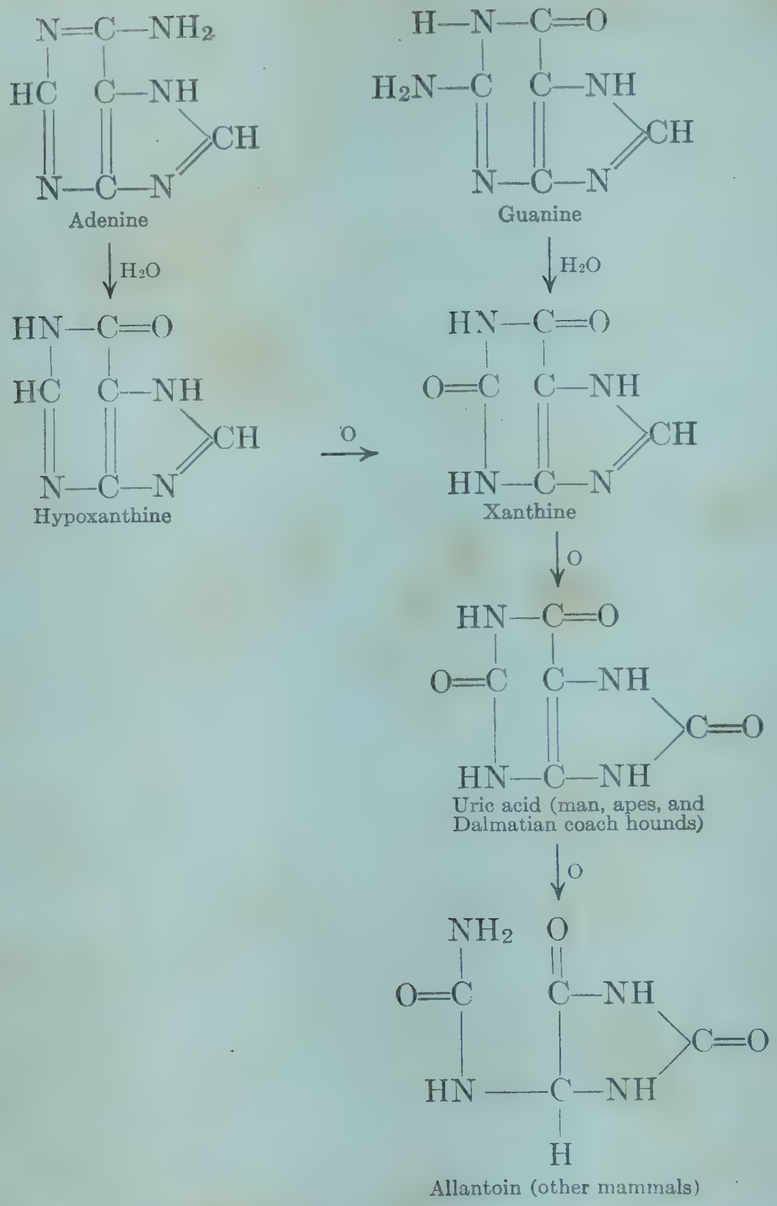
thesis of phospholipids and the intermediates in carbohydrate metabolism. Some may be deposited in the bones as calcium phosphate. Any excess is eliminated in the urine as inorganic phosphates.

Metabolism of Purines. Of especial interest in nucleic acid metabolism is what happened to the pyrimidines and the purines. Little is known about the metabolism of the pyrimidines. Under normal conditions they appear to be converted into urea. When fed in large quantities, they are largely excreted in the urine unchanged.

The two purines found in nucleic acid are **adenine** and **guanine.** Adenine is 6-aminopurine, and guanine is 2-amino-6-oxypurine. In metabolism, the amino groups are removed from the purines by a process of hydrolysis, leaving oxypurines. Adenine hydrolyzes to **hypoxanthine** (6-oxypurine), and guanine to **xanthine** (2,6-dioxypurine). Hypoxanthine is also oxidized to xanthine. Finally xanthine is oxidized to **uric acid** (2,6,8-trioxypurine), which is eliminated in the urine. Uric acid is the end product of purine metabolism in man, anthropoid apes, and Dalmatian coach hounds. In other mammals the oxidation goes a step further to form **allantoin.** The outline on p. 278 shows the course of purine metabolism.

What has just been said applies to the purines derived from food. Purines are also being formed continuously from the decomposition of nucleoproteins in the tissues. In man these purines are also converted into uric acid, which is excreted in the urine. Thus the uric acid in the urine is derived from both exogenous and endogenous sources.

Synthesis of Purines and Pyrimidines. The question next arises of whether an animal can synthesize purines and pyrimidines for the building of the nucleoproteins of the tissues. There is considerable evidence to support the view that the animal body has this ability. Individuals fed on a very low purine diet gain in weight and excrete more uric acid in the urine than can be accounted for by the purines in


Adenine
H_2O
Hypoxanthine
O
Guanine
H_2O
Xanthine
O
Uric acid (man, apes, and Dalmatian coach hounds)
O
Allantoin (other mammals)

the food. During the incubation of an egg the purine content increases, indicating a synthesis of purines from nonpurine material. The main nitrogenous constituent of the excreta of birds and reptiles is uric acid. In other words, uric acid in birds and reptiles takes the place of urea in man. If birds and reptiles can convert most of their nitrogenous waste to uric acid, they certainly have the power of synthesizing purines; and it is reasonable to suppose that man can synthesize at least the purines necessary for the production of his nucleoprotein requirements.

It is thought that histidine may give rise to purines in the body. When histidine is fed to animals, the amount of allantoin excreted in the urine is increased. Isotope studies indicate that the uric acid in the excretion of birds comes from nucleic acid and that arginine and urea are not involved in its synthesis.

Sulfur Metabolism. Sulfur is an important constituent of the body. It is derived largely from protein, being present in the amino acids **cystine** and **methionine.** Rose has shown that methionine is essential in the diet and that cystine is not. It is believed that methionine may be converted into cystine in the body, but that the reverse reaction does not take place. Thus methionine may replace cystine in the diet, but cystine cannot replace methionine.

Sulfur is found widely distributed in the body. In human hair there is about 15 per cent of cystine. In glutathione it occurs in the form of cysteine. In the bile it is presented in taurine, a constituent of one of the bile salts. As sulfuric acid it appears in certain glycoproteins, such as the mucin of the saliva and the proteins of the cartilages. It is also found in insulin and in Vitamin B_1, where it evidently plays a part in biological oxidations.

When the sulfur-containing amino acids are oxidized in the body, the sulfur is largely converted into sulfates. The sulfate radical may unite with metallic elements to form **inorganic sulfates,** or it may be conjugated with organic radicals to form **ethereal sulfates.** These sulfates are excreted in the urine, and the sulfur is known as **oxidized sulfur.** The amount of oxidized sulfur in the urine varies with the protein intake and in this respect is like urea.

Not all the sulfur in the body is oxidized before being excreted. A rather constant amount, known as **neutral sulfur,** is found in the urine in the unoxidized form. In this respect neutral sulfur is like creatinine. The neutral sulfur of the urine is composed of a variety of sulfur-containing compounds, a few of which are cystine, methyl mercaptan, ethyl sulfide, thiocyanates, and taurine derivatives. In some individuals it may consist largely of cystine, in which event the condition is known as **cystinuria.** Under certain conditions cystine may form deposits in the kidney, with serious consequences.

Ketogenic and Antiketogenic Amino Acids. Several of the amino acids are similar to alanine in that they are capable of giving rise to glucose in the animal body. They are called the **glycogenic** or **antiketogenic** amino acids. Glycine, alanine, serine, cystine, aspartic acid, glutamic acid, hydroxyglutamic acid, arginine, and proline are examples.

Some amino acids appear to be oxidized like fats rather than like carbohydrates. In diabetic animals they give rise to acetoacetic acid and are said to be **ketogenic.** Leucine, phenylalanine, tyrosine, and hydroxyproline are ketogenic amino acids. Lysine and valine appear to be neither ketogenic nor antiketogenic, so that they give rise to neither acetoacetic acid nor glucose as a result of their metabolism.

Essential and Nonessential Amino Acids. Much work has been done on the importance of the various amino acids in nutrition. Rose and his co-workers have made an exhaustive study of the known amino acids and their relation to growth of rats. In the course of their work they discovered a new amino acid, α-amino-β-hydroxy-*n*-butyric acid, which they found to be essential for growth. They named this new amino acid **threonine,** because it has the same configuration as threose, the tetrose sugar. It should be noted that Rose's work dealt only with the amino acid requirements for growth. What the requirements may be for other physiological functions, such as reproduction or lactation, cannot be stated at the present time. However, it appears that all the amino acids are required for growth and that certain ones are apparently nonessential because the body is able to synthesize them. If the body is able to synthesize an amino acid for growth, supposedly it can also synthesize the amino acid for any other biological need.

According to Rose, the amino acids may be classified according to their nutritive importance for the growth of rats as follows:

Essential	Nonessential
Lysine	Glycine
Tryptophan	Proline
Methionine	Hydroxyproline
Histidine	Aspartic acid
Phenylalanine	Glutamic acid
Isoleucine	Hydroxyglutamic acid
Valine	Serine
Leucine	Alanine
Threonine	Norleucine
Arginine	Cystine
	Tyrosine
	Citrulline

In these lists arginine is classified as an essential amino acid. If arginine is excluded from the diet, growth occurs but not at a normal rate. Hence it is said that arginine is essential for normal growth.

Rose has also studied the amino acid requirements of man. He found that they are the same as those for rats with two exceptions. Adult human subjects do not require arginine or histidine. Table 9 gives the minimal daily requirements for those amino acids that are indispensable in the diet of normal men. Rose recommends that twice these quantities be consumed daily in order to insure an adequate supply of these amino acids in the diet.

TABLE 9

MINIMAL AND RECOMMENDED INTAKES OF ESSENTIAL AMINO ACIDS FOR NORMAL MEN

Amino acid	Minimal daily requirement, gm.	Recommended daily intake, gm.
L-Tryptophan	0.25	0.50
L-Phenylalanine	1.10	2.20
L-Lysine	0.80	1.60
L-Threonine	0.50	1.00
L-Valine	0.80	1.60
L-Methionine	1.10	2.20
L-Leucine	1.10	2.20
L-Isoleucine	0.70	1.40

Holt and Albanese have also studied the amino acid requirements of man. They believe that arginine is an essential amino acid. When it is lacking in the diet of man, there is a marked decrease in the number of spermatozoa produced; in rats they noted marked tissue changes in the testes. Thus arginine appears to be essential for reproduction in the male. Holt and Albanese also suggested that histidine may be essential in the diet of man.

The amino acid requirement of other animals has been studied by several workers. Bauer and Berg found that mice require the same amino acids as rats except that arginine is not required for maximum growth. Almquist has shown that glycine is indispensable in the diet of chicks. Elvehjem and co-workers found that arginine, glutamic acid, cystine, and, under certain conditions, tyrosine, are also required by chicks. Rose and Rice found that dogs required the same amino acids as rats with the exception of arginine which was dispensable.

Biological Value of Proteins. We have just learned that certain amino acids cannot be synthesized by the body and therefore are essential in the diet. Since the various proteins found in foods differ in their amino acid content, it is obvious that they should differ in their usefulness as foods. The most important function of protein in nutrition is to supply the amino acids necessary for the building and maintenance of body tissue. The percentage of absorbed protein in a food that is utilized by the body for the building and maintenance of body tissues is called its biological value.

In calculating the biological value of proteins nitrogen values are used. The amount of absorbed nitrogen (AN) is equal to the nitrogen intake (NI) minus the difference between the amount of fecal nitrogen (FN) on the diet being tested and the fecal nitrogen on a protein-free diet (FN^1). This may be expressed in the following formula:

$$AN = NI - (FN - FN^1)$$

The biological value (BV) may then be determined with the following formula:

$$BV = \frac{AN - (UN - UN^1)}{AN} \times 100$$

In the above formula UN is the urinary nitrogen on the diet being tested, and UN^1 is the urinary nitrogen on a protein-free diet.

Assuming the following data, the calculation would be as follows:

mg.

$$NI = 742$$
$$FN = 75$$
$$FN^1 = 50$$
$$UN = 613$$
$$UN^1 = 298$$
$$AN = 742 - (75 - 50) = 717$$
$$BV = \frac{717 - (613 - 298)}{717} \times 100 = 56$$

Mitchell obtained the following biological values for proteins found in some common foods:

Food	Biological Value
Whole egg	94
Milk	85
Egg white	83
Beef liver	77
Beef round	69
Whole wheat	67
Potato	67
Rolled oats	65
Wheat flour	52
Navy beans (cooked)	38

The method described above for determining the biological value of a protein takes into account the requirements for both growth and maintenance.

In determining the biological value of proteins, it is important that the diet supply only the amount of protein necessary for growth and maintenance. If more protein is fed, any excess will be oxidized for energy and low biological values will be obtained. A level of 10 per cent protein in the diet is often used in these studies.

Supplementary Value of Proteins. From the table presented it is evident that proteins from different sources vary in their biological values. The reason for this is that they differ in their amino acid content. The proteins of eggs contain about the right quantity of each essential amino acid for growth and maintenance, whereas those in navy beans do not. Each protein that has a low biological value must have a limited supply of one or more essential amino acids. If one feeds a mixture of two proteins each of which has a low biological value when fed alone, the biological value of the combination is higher if one protein supplies the essential amino acids that are lacking in the other. The value of proteins for supplying essential amino acids which are lacking in other proteins is called their supplementary value. If a protein has the proper assortment of amino acids for tissue building, it is said to have very high biological value, but it is of little value in supplementing a protein of poor biological value. In general, it may be said that the lower the biological value of a protein when fed by itself, the higher is its supplementary value when used with a protein lacking the amino acid in which it is high.

Mitchell and Kick fed pigs on a mixture of corn and tankage. The corn protein had a biological value of 54 and that for tankage was 42. The combination (2 : 1) had a biological value of 61. In general it may be stated that animal proteins are a good supplement for vegetable proteins.

Amino Acids in Medicine. In recent years much use has been made of proteins and protein hydrolysates in surgery and in the treatment of starvation and burns. People who have starved for a long time lose their appetites, and their digestive systems are so upset that they cannot eat and, even if they could, would be unable to digest their food. It has been found that feeding hydrolyzed protein in the form of a solution either by a stomach tube or by intravenous injections carries such patients over the crisis until they can take food in the normal manner. After World War II many lives were saved by this method among victims of prison camps.

In civilian life it is often found that patients who appear in hospitals for major operations are suffering from protein starvation due to loss of appetite. Often after an operation a patient loses considerable weight because of the limited amount of food that he is able to take. It has been found that surgical patients do much better if they are fed hydrolyzed protein before and after an operation. The period of convalescence is much shortened.

It has been shown that the ability of the body to resist infection is related to the concentration of a protein called γ-globulin in the blood.

The antibodies of the blood either are the γ-globulin fraction or are associated with it. γ-Globulin has been used to protect children against infantile paralysis. Animals that have been on a low-protein diet have less γ-globulin in the blood and are more susceptible to disease than animals on a high-protein diet. Thus it appears that our state of protein nutrition is an important factor in disease resistance.

Perhaps the most spectacular use of protein hydrolysates has been in the treatment of severe burns. One of the hazards of such a burn is that large amounts of protein material are lost from the body in the exudate which oozes out over the surface of the burn. If this protein loss can be compensated for by injecting large quantities of hydrolyzed protein, there is reasonable hope for the recovery of the patient.

In many of these cases involving intravenous injection, blood plasma may be used in place of protein hydrolysates. Because of the difficulty of getting sufficient human plasma when large quantities are required, protein hydrolysates made for casein are more practical to use. Unhydrolyzed proteins, other than those of human plasma, usually cannot be used because of the allergic reactions which they induce. One disadvantage of using protein hydrolysates is that they are very unpalatable and must be administered by stomach tube or by vein.

REVIEW QUESTIONS

1. Name two important functions of amino acids in nutrition.
2. What is meant by the term nitrogenous equilibrium?
3. What is meant by a negative and by a positive nitrogen balance?
4. What evidence is there that proteins are stored in the body?
5. In what form and in what organs are proteins stored?
6. What are isotopes? How are they used in studying protein metabolism?
7. When isotopic leucine is fed, where is the heavy nitrogen found in the tissues? What is the isotope content of glutamic acid and of lysine?
8. What is meant by transamination?
9. Indicate two ways by which amino acids may be synthesized in the body.
10. How may carbohydrates be converted into amino acids in the body?
11. What evidence is there that the carbon chain of one amino acid may be converted into the carbon chain of another?
12. Name the four most important nitrogen compounds found in the urine.
13. State the Krebs and Henseleit theory of urea formation. In what organ is urea formed?
14. What is the origin of urinary ammonia?
15. From what three sources is creatine derived?
16. What is the origin of creatinine?
17. Name the pyrimidines and the purines found in nucleoprotein.
18. What is the end product of pyrimidine metabolism?
19. What is the end product of purine metabolism in man? In other mammals?
20. What is the precursor of uric acid in metabolism?

21. What evidence is there that purines are synthesized in the body?

22. Discuss sulfur metabolism. What important amino acids contain sulfur? Distinguish between inorganic sulfates, ethereal sulfates, and neutral sulfur in the urine.

23. What is meant by a ketogenic and by an antiketogenic amino acid? Give examples of each type.

24. Name the essential and nonessential amino acids for man.

25. What is meant by the biological value of a protein? How is it determined?

26. Discuss the supplementary value of proteins.

27. How are amino acids and protein hydrolysates used in medicine?

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15

Calorimetry

Since the human body is as a rule warmer than its environment, it is obvious that heat must be supplied to the body in order that it may maintain its temperature. It is obvious also that energy must be supplied for the mechanical work which the body does. This heat and mechanical energy come from the oxidation of the foods which are eaten. In this chapter we shall consider the various food materials as sources of heat and mechanical energy.

Regulation of Body Temperature. The normal temperature of the human body is about 37°C. or 98.6°F., when taken by mouth. Rectal temperatures are slightly higher than this, and temperatures taken under the arm are slightly lower. During a 24-hour period the body temperature fluctuates slightly; it is highest at 5 P.M. and lowest at 5 A.M. The normal range of body temperature is from 96.7° to 99°F. when taken by mouth. The fact that body temperature fluctuates so little indicates the presence of an efficient thermostatic control. Many animals often spoken of as cold-blooded have temperatures close to that of their environment. When their environment becomes cold, they lose their activity.

In the higher animals body temperature is controlled by a certain part of the brain, often spoken of as the **heat center.** A fever is usually due to the heat center being affected by toxins produced by bacteria. Certain drugs lower body temperature by the effect, the opposite to that of bacteria, which they have on the heat center. Infants must be protected from exposure because their heat-regulating mechanism is poorly developed.

Practically, body temperature is controlled by regulating heat production or heat loss from the body. When a person is cold, exercise will make him warm. Shivering may be looked upon as involuntary exercise. On a hot day refraining from exercise aids in keeping cool.

The most important means of regulating body temperature is by controlling heat loss. Since water has a high heat of vaporization, any water which evaporates from the body removes heat. In warm weather our sweat glands open and we perspire. As the sweat evaporates from the surface of our bodies, there is a cooling effect. Fans aid in keeping us cool by hastening evaporation. Moisture is also lost from our bodies through our lungs when we breathe. The importance of this method of losing heat from the body is seen in dogs, which pant in hot weather. Dogs do not sweat but lose much heat from the body through evaporation by way of the mouth. Since evaporation is related to humidity, it is obvious why a high temperature is not as oppressive in a dry climate as in a humid one.

Heat is continually being lost from the body by direct radiation. The temperature of the surface of the body varies considerably, depending upon the temperature of the environment. The amount of heat lost from the body by radiation depends upon the difference between the temperature of the body and that of its environment. In cold weather the skin becomes cold because there is a restriction of the blood supply to the skin. Thus heat loss is lessened. In warm weather blood comes to the surface, and the skin is warm, thus aiding in heat loss by radiation. A person becomes flushed when the temperature is high.

Calories. Heat is measured in terms of calories. A **small calorie** is the amount of heat required to raise the temperature of 1 gram of water from 15° to 16°C. Since this unit is small, it is customary to use a larger one in animal calorimetry. This unit is the **large Calorie,** which is equivalent to 1000 small calories. In working with domestic animals such as the cow, a still larger unit is sometimes used, called the **therm,** which is equivalent to 1000 large Calories. Since heat energy may be converted into mechanical energy, it is possible to express mechanical energy in terms of calories. One large Calorie is equivalent to 3087 foot-pounds. A **foot-pound** is the amount of energy required to raise a weight of 1 pound a distance of 1 foot against the force of gravity.

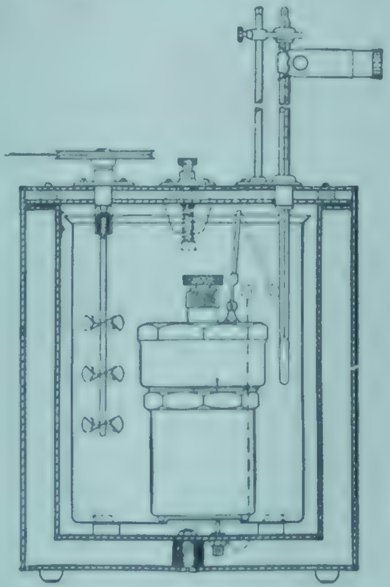

FIG. 22. Cross section of a Burgess-Parr oxygen bomb calorimeter.

Gross Energy. The heat of combustion of a food may be determined by burning a known weight of the food in a bomb calorimeter. (See Fig. 22.) Essentially this is a hollow metal chamber in which is placed a platinum boat containing the sample to be analyzed. The chamber is filled with oxygen under pressure, and the sample is ignited electrically. During combustion the bomb is submersed in a known volume of water. From the rise in temperature of the water the number of calories of heat generated may be calculated. The amount of energy obtained from a food when completely burned in a bomb calorimeter is known as its gross energy.

Metabolizable Energy. When foods are consumed the body is unable to utilize all of the energy which they contain. Some of the food may not be digested or absorbed and will appear in the feces; that which is absorbed may not be completely oxidized in the body and appear in the urine as partially oxidized organic compounds; some is acted upon by intestinal bacteria, forming gases such as methane, which is of no use to the body. If one determines the energy in the feces, urine, and intestinal gases and subtracts the sum of these values from the gross energy of the food consumed one obtains the metabolizable energy of the food. This may be defined as the energy that the body is able to derive from a food.

The following table gives the number of large Calories obtained when 1 gram of the various food constituents is burned in a bomb calorimeter and when it is metabolized in the body. It also shows the percentage of the gross energy of each food constituent utilized by the body.

	Gross Energy, Calories	Metabolizable Energy, Calories	Percentage Utilized by the Body
Carbohydrate	4.19	4.0	95.4
Protein	5.65	4.0	70.8
Fat	9.54	9.0	94.3

It can be seen from the table that both carbohydrates and fats are fairly well utilized by the body. The slight loss can be accounted for by incomplete absorption of these food constituents or by the excretion in the urine of small amounts of intermediate products of metabolism which have not been completely oxidized. Proteins are not as completely oxidized in the body as carbohydrates or fats. The nitrogen of proteins is eliminated in the urine as nitrogenous organic compounds, such as urea, uric acid, or creatinine, which may be oxidized with the liberation of heat. Carbohydrates and fats are oxidized almost completely to carbon dioxide and water, which are the final products of oxidation. Gram for gram, carbohydrates and proteins have about the same energy values when oxidized in the body, whereas fats produce about two and one-fourth times as much energy as carbohydrates. When the formulas for carbohydrates and fats are considered, it is at once evident why fats give more energy on combustion than carbohydrates. Carbohydrates have much oxygen in the molecule; fats have little. Thus carbohydrates are partly oxidized to begin with, and less heat would be expected to be evolved during the complete oxidation of such a compound than during the oxidation of a fat, which contains little oxygen.

Animal Calorimetry. The first work on the measurement of heat production by the animal body was done by Lavoisier. He used an **ice calorimeter,** in which the chamber containing the animal under investigation was packed in ice. The heat produced by the animal melted the ice, and from the amount of water produced the heat given off by the animal was calculated. Today **respiration calorimeters** are used. The best work in animal calorimetry has been done by Rubner in Germany, experimenting with small animals; Atwater, Rosa, and Benedict in this country, with humans; and Armsby, with the larger farm animals.

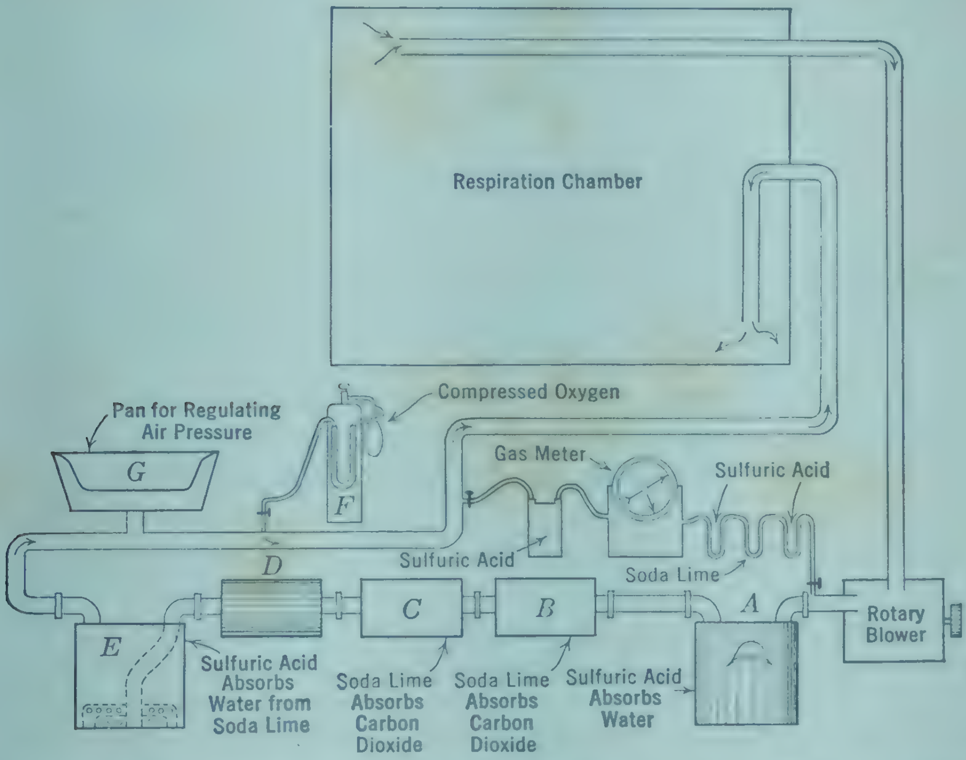
Respiration Chamber
Compressed Oxygen
Pan for Regulating Air Pressure
G
F
Gas Meter
Sulfuric Acid
Sulfuric Acid
Soda Lime
D
C
B
A
Rotary Blower
E
Sulfuric Acid Absorbs Water from Soda Lime
Soda Lime Absorbs Carbon Dioxide
Soda Lime Absorbs Carbon Dioxide
Sulfuric Acid Absorbs Water

FIG. 23. Diagram of Atwater-Rosa-Benedict respiration calorimeter for human beings. (After Benedict and Milner.) From *Physiological Chemistry* by Mathews.

A respiration calorimeter (see Fig. 23) consists of a well-insulated chamber with three walls separated by dead air spaces. By electrical heating coils and cold-water pipes distributed in the dead air spaces the spaces may be maintained at the same temperature as the inner chamber, and thus there is no loss or gain in heat through the walls of the chamber. Inside the chamber are pipes through which water may be circulated to remove the heat produced by the animal. The temperature of the ingoing and outgoing water and its volume being known, the calories of heat removed may be calculated. Through the chamber, air is circulated; the outgoing air is passed through sulfuric acid to remove water vapor and through soda lime to remove carbon dioxide. As the oxygen is removed from the air by the animal in the chamber, more is added from a tank of compressed oxygen. Since the water vapor given off by the animal requires heat to volatilize it, the weight of water produced must be measured. This is determined by noting the increase in weight of the sulfuric acid container. The latent heat of vaporization of this water in calories added to the calories removed from the chamber by the water going through the cooling pipes indicates the total heat produced by the animal. From the increase in weight of the soda lime container and the loss in weight of the oxygen tank, the carbon dioxide production and oxygen consumption can be determined. In most calorimeters provision is made for introducing food and removing excreta. Therefore special precautions must be taken to prevent error due to the introduction or removal of heat from the chamber. The food entering the chamber and the excreta leaving it must have the same temperature as the chamber or proper corrections must be applied. In calorimeters for human beings provision is made for the individual to operate a bicycle connected with an instrument for measuring the amount of work done. Thus the food requirement for doing work may be studied.

Respiratory Quotient. The respiratory quotient, usually spoken of as the **R.Q.**, is the **volume** of carbon dioxide produced divided by the **volume** of oxygen consumed by an animal. According to Avogadro's law, equal volumes of all gases at the same temperature and pressure contain the same number of molecules. Hence the R.Q. may be considered the number of molecules of carbon dioxide produced divided by the number of molecules of oxygen consumed.

When glucose, a typical carbohydrate, is completely oxidized, the following reaction occurs:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Applying Avogadro's law, we can say that, when sugar is oxidized, six volumes of carbon dioxide are produced when six volumes of oxygen are consumed:

$$\text{R.Q.} = \frac{6}{6} = 1$$

Thus the R.Q. when sugar is completely oxidized in the body is 1.

When a typical fat like tristearin is oxidized completely, the following reaction takes place:

$$2C_{57}H_{110}O_6 + 163O_2 \rightarrow 114CO_2 + 110H_2O$$

Hence

$$\text{R.Q.} = \frac{114}{163} = 0.7$$

With proteins the calculations are more complicated, since proteins are not completely oxidized to carbon dioxide and water in the body. The R.Q. when proteins are oxidized in the body is 0.8. Since under ordinary conditions an animal is oxidizing some of all three of the foodstuffs, the R.Q. is between 0.7 and 1.0. As a rule the R.Q. during rest is about 0.82.

When an animal is eating an excess of carbohydrate and converting the excess to fat, the R.Q. is more than 1. This fact can readily be understood when it is considered that carbohydrates contain more oxygen than fats. When carbohydrates are changed to fats, oxygen becomes available for oxidation purposes, and less oxygen is consumed from the air for carrying on the oxidation processes in the body.

In diabetes the R.Q. may approach 0.7 indicating that fats are being metabolized rather than sugar and protein. Low R.Q. values have also been noted during hibernation when animals are living on stored fat.

If the oxygen consumption, the carbon dioxide production, and the amount of nitrogen in the urine are known for a given period, it is possible to tell how much of each food constituent has been oxidized during the period. From the urinary nitrogen the amount of protein oxidized, and therefore the amount of oxygen and carbon dioxide involved in its oxidation, can be calculated. By subtraction the amount of oxygen and carbon dioxide involved in the oxidation of nonprotein material can be found, and thus the nonprotein R.Q. can be determined. Once the nonprotein R.Q. is known, the weight of carbohydrate and fat equivalent to each liter of oxygen consumed can be calculated. Tables are available which makes this calculation very simple.

Basal Metabolism. The rate of oxygen consumption, carbon dioxide formation, and heat production is influenced by various factors, such as muscular activity, digestion, and emotional excitement. The rate of heat production of an individual during physical, emotional, and digestive rest is known as his **basal metabolic rate.** Since during rest the respiratory quotient is fairly constant, there is a rather definite correlation between heat production, oxygen consumption, and carbon dioxide production. As the direct measurement of heat produced by an individual is a complicated procedure, it is customary to determine the basal metabolic rate indirectly by measuring the oxygen consumed in a given length of time. Each liter of oxygen consumed is equivalent to 4.825 Calories, assuming the R.Q. to be 0.82, a value that has been found for a person who is at rest and who has not eaten recently. A person whose basal metabolic rate is to be determined should be given a light supper the night before the test and should have a good night's rest. The test is run in the morning before breakfast, preferably shortly after awakening.

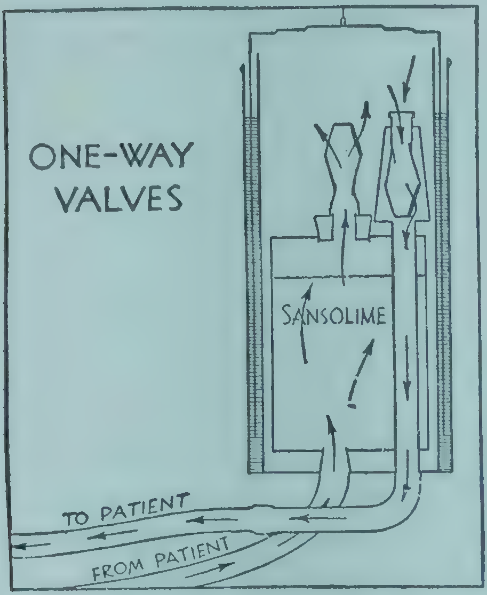

Fig. 24. Cross section of Sanborn graphic metabolism apparatus. Courtesy of the Sanborn Co.

The apparatus (see Fig. 24) consists of a metallic bell jar filled with pure oxygen and sealed with water. By means of rubber tubes the pure oxygen is inhaled. The exhaled gases pass through soda lime to remove carbon dioxide, before being returned to the bell jar. Thus the loss in volume of oxygen in the bell jar indicates the volume of oxygen consumed. The test requires from 8 to 10 minutes for completion. During the test the apparatus draws a line on a special chart by means of which the volume of oxygen consumed per minute may be read directly. The result obtained is compared with normal values and is expressed in percentage above or below normal. The normal values are taken from tables which have been compiled from actual tests on healthy individuals of both sexes and of various ages, weights, and heights.

Influence of Surface Area. It is evident that the heat produced by the metabolic processes of the body is mainly lost by radiation from the surface. Therefore surface area is the most important factor determining the basal metabolic rate of an animal. It has been found that the heat produced during rest by all animals is approximately the same when expressed in terms of surface area. About 1000 Calories are produced per square meter per day. Thus small animals produce much more heat on a unit of weight basis than large animals. A mature human male produces 39.5 Calories per square meter per hour, and a mature female 36.5 to 37 Calories. Surface area may be calculated from the following formula:

$$A = W^{0.425} \times H^{0.725} \times 71.84$$

where

A = area in square centimeters.
W = weight in kilograms.
H = height in centimeters.

From the foregoing discussion it is apparent that weight and height are important factors in estimating an individual's normal basal metabolic rate.

Influence of Age. Age is an important factor affecting the basal metabolic rate. A newborn baby produces from 600 to 700 Calories per square meter of surface per day, as compared to 1000 Calories for an adult. During the first few years of life the basal metabolic rate rises rapidly, reaching a value 20 per cent above that of the adult. After puberty there is a gradual decline. A 15-year-old male produces 46 Calories per square meter per hour; at 30 years the number is reduced to 39.5 Calories; and at 70 years, to 35.5 Calories. A female produces from 2.5 to 3.0 Calories less at corresponding ages.

Diagnosis of Disease. The determination of the basal metabolic rate has found wide application in hospitals in the diagnosis of certain diseases, especially goiter, a disease of the thyroid gland. In **exophthalmic goiter** basal metabolic rates from 20 to 50 per cent above normal are common, and in severe cases they may be much higher. In **cretinism,** a disease associated with an underdeveloped thyroid, basal metabolic rates of 20 per cent below normal are common. In **leukemia** values 20 per cent above normal may be expected. **Fevers** have been shown to increase the basal metabolic rate. A 1° C. rise in temperature increases the rate 13 per cent. For this reason it is very important to know a person's temperature at the time a basal metabolism test is made. If it is above or below normal, corrections should be made.

During **starvation** the basal metabolic rate decreases. In one instance a man's basal metabolic rate decreased 26 per cent after 21 days of fasting.

Specific Dynamic Action of Foods. The test for determining the basal metabolic rate is made during digestive rest, because it has been found that shortly after the ingestion of food the metabolic rate is increased, especially after a protein meal. This rise in metabolic rate after the digestion and absorption of food is said to be due to the **specific dynamic action** (S.D.A.) of food. Sometimes this is referred to as **heat increment,** a term suggestive of what S.D.A. really is.

The various food constituents show different specific dynamic effects. Proteins show the greatest effect. If an animal is fed the number of calories required for its basal metabolism in the form of protein, the amount of heat produced will be 30 per cent greater than that represented by the food consumed. With carbohydrate it will be about 6 per cent and with fat about 4 per cent greater. The extra heat comes from the oxidation of foods stored in the body. When all three food constituents are fed the effect is not the sum of each but is less. Forbes and Swift fed rats a mixture of beef protein and lard and found that the specific dynamic effect of the combination was less than for the lard alone and about one-half that calculated for the sum. They point out that under certain conditions fats may be more important than proteins in determining the specific dynamic effect of food mixtures. In terms of total calories of the food used for maintenance purposes it has been estimated that on a mixed diet about 10 per cent must be added to take care of the specific dynamic effect.

Many theories have been suggested to explain this specific dynamic action of foods, but as yet no theory has been advanced which is entirely satisfactory. It is possible that simply the excess supply of food material present in the tissues after a meal speeds up oxidative reactions by its mass effect. The high effect from proteins has been attributed to a stimulatory effect of certain amino acids on cellular metabolism. Amino acids themselves stimulate metabolism, although, it is interesting to note, the various amino acids differ in their ability to do so. Thus glycine, alanine, and phenylalanine have a very high specific dynamic effect, whereas leucine and tyrosine have little effect. Work by Kriss indicates that the effect of amino acids is due to the metabolism of the carbon chains of the various amino acids.

Borsook believes that the specific dynamic action of proteins may be accounted for by the heat generated during the various stages of amino acid metabolism. Heat is generated when amino acids are deaminized, when urea is formed from ammonia and carbon dioxide in the liver, and when urea is excreted by the kidney. Also, if the carbon chain of the amino acid is converted into glycogen, heat is generated.

From the above discussion it is evident that after eating one produces more heat than is necessary for maintaining a normal body temperature under ordinary conditions. In hot weather the eating of a heavy meal adds to one's discomfort. The drinking of cold soft drinks, which contain sugar, or the eating of ice cream to cool off may bring temporary relief but eventually increases heat production. It would be much better simply to drink cold water. In cold weather the stimulation of metabolism is an advantage since the extra heat produced is used to maintain a normal body temperature, thus conserving stored food supplies.

Net Energy Value of Foods. From what has been said in the previous section it is evident that a considerable proportion of the energy in a food is lost in the form of heat over and above that required to maintain a normal body temperature. This heat is wasted and is of no value to the body. Armsby, working with farm animals, suggested that the energy lost from a feed because of its specific dynamic action should be subtracted from its metabolizable energy in order to express its true value as a feed. He called this new value the net energy value of a feed. The net energy value of a feed may be defined as the energy in a feed which is used for maintenance, growth, and the production of a product such as milk or eggs. Armsby determined this net energy by feeding at a certain level and measuring the heat produced. He then increased the amount fed and again measured the heat produced. The increase in calories produced subtracted from the metabolizable energy of the extra feed gave the net energy contained in that amount of feed.

Source of Energy for Work. We have seen that energy must be supplied to the body for its functioning under basal conditions and that, in addition, from 10 to 15 per cent is required to take care of the specific dynamic action of the food eaten. If the body is to do work, energy in the form of food must be supplied for this purpose. An interesting question arises as to the source of the mechanical energy used by the body. At one time it was thought that the proteins of the tissues were oxidized during muscular activity and that a high-protein diet was necessary for a working man. We now know that violent muscular activity has little effect on protein metabolism. The amount of nitrogen excreted in the urine is about the same whether a person is resting or working hard.

If a person at hard work is supplied with an adequate amount of all three foodstuffs, the respiratory quotient approaches 1.0, an indication that carbohydrates are the choice of the muscles as their source of energy. If necessary, however, any of the three foodstuffs may be utilized for energy production, and their relative values may be calculated from their metabolizable energy equivalents.

Since under basal conditions the R.Q. is 0.82, it is evident that under these conditions carbohydrates, fats, and proteins are all being oxidized in the body. Since work is being done in the body even at rest by such organs as the heart, it appears that any foodstuff may be a source of energy for mechanical work.

Mental effort apparently has little influence on energy metabolism. The slight increase of 4 per cent in metabolism found during intense mental activity may be accounted for by the involuntary muscular activity of organs like the heart and lungs.

Energy Requirements in Nutrition. In calculating the energy requirements for an adequate diet, all the factors just discussed must be considered. From basal metabolism studies the number of Calories necessary for maintenance is known. To this figure must be added sufficient Calories to take care of the specific dynamic action of the food consumed and the work of digestion. Finally, the food must supply the energy for work.

The Food and Nutrition Board of the National Research Council recommends the following daily energy allowances for humans.

DAILY ENERGY ALLOWANCES FOR HUMANS

Children up to 12 Years	Calories
Under 1 year	100 per 2.2 lb.
1–3 years (29 lb.)	1200
4–6 years (42 lb.)	1600
7–9 years (55 lb.)	2000
10–12 years (75 lb.)	2500

Boys	Calories	Girls	Calories
13–15 years (103 lb.)	3200	13–15 years (108 lb.)	2600
16–20 years (141 lb.)	3800	16–20 years (119 lb.)	2400
Men (154 lb.)		Women (123 lb.)	
Sedentary	2400	Sedentary	2000
Moderately active	3000	Moderately active	2400
Very active	4500	Very active	3000
		Pregnancy (latter half)	2400
		Lactation	3000

In arranging diets for children it should be borne in mind that during childhood the basal metabolic rate is higher than that of the adult. The great physical activity of a child must also be considered. Finally, excess food must be provided for growth. A child of 2 years requires about 1100 Calories; a boy of 12 years, from 2300 to 3000 Calories.

REVIEW QUESTIONS

1. What is the normal temperature of the body? How does the body maintain this temperature?
2. Define small calorie, large Calorie, therm, and foot-pound.
3. What is the mechanical equivalent of 1 Calorie?
4. Define gross energy and metabolizing energy of a food.
5. Describe a respiration calorimeter.

6. Define respiratory quotient. What do the following R.Q.'s indicate: 1.0, 1.2, 0.7, 0.8, and 0.82?

7. Knowing the R.Q. and the amount of nitrogen in the urine, how can one determine the amounts of carbohydrate, fat, and protein being metabolized in the body?

8. What is meant by basal metabolic rate? How is it determined, and of what value is it in the diagnosis of disease?

9. How many Calories are produced per square meter of body surface per day?

10. How do age, sex, and body temperature influence the basal metabolic rate?

11. What is meant by the specific dynamic action of foods? Are all foods equal in their specific dynamic action?

12. What is meant by the net energy value of a food?

13. What class of foods is ordinarily used by the body for work? What evidence can be given in support of your answer?

14. What are the daily energy requirements of humans for various ages and activities?

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16

Composition of Tissues

The body consists of several types of tissues, which differ in their composition. In this chapter we shall discuss briefly the chemical characteristics of some of the more important tissues.

Muscle Tissue. The muscles may be considered the motor organs of the body. Most muscles are attached to the skeleton; and, when they contract, they cause motion in the body. These muscles are under the control of the will and are said to be voluntary. Microscopically, **voluntary muscles** have a striated appearance and are sometimes called **striated muscles.**

In contrast to the voluntary muscles is a group of muscles that are not under the control of the will. They are called **involuntary muscles** and are represented by such muscles as those of the stomach and intestinal walls and the blood vessels. Involuntary muscles are not striated and are often referred to as **unstriated** or **smooth muscles.**

A third type of muscle, known as **cardiac muscle,** is found in the wall of the heart.

Our knowledge of the composition of muscle tissue has been obtained mainly from the study of voluntary muscles, such as those found in lean meat. Such muscles contain about 75 per cent of water and 25 per cent of solids. About 80 per cent of the solids is protein, the remainder being materials called extractives, which are soluble in water, alcohol, or ether. Among the extractives are found lipids, carbohydrates, mineral salts, and a group of miscellaneous compounds, many of which are nitrogenous.

Muscle Proteins. Voluntary muscles are composed of fibers made up of many fibrils arranged parallel to each other and to the axis of the fiber. These fibrils are bathed in a liquid called sarcoplasm. The fibrils are composed of a globulin called **myosin.** In the sarcoplasm another globulin is found, known as **globulin X,** and an albumin known as **myogen.** A fourth protein, known as **myoglobin** is also present in muscles.

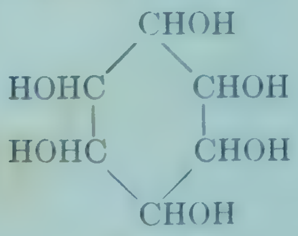
Myosin is the protein in muscles that contracts. Myosin is thought by some to be an enzyme which hydrolyzes adenosinetriphosphate, forming adenosinediphosphate and phosphoric acid, thereby liberating energy from a high-energy phosphate bond for muscular contraction. Others believe that a special enzyme, closely associated with myosin, is involved.

Szent-Györgyi believes that the protein of muscle fibrils is a combination of two proteins, actin and myosin, and he calls the combination **actomyosin.** Neither actin nor myosin alone will contract but actomyosin will. The terms myosin and actomyosin are often used interchangeably.

By submitting muscle to high pressure, a liquid may be obtained, called **muscle plasma,** which clots on standing in a manner similar to blood. This clotting is caused by changes in muscle proteins, especially myosin. It is believed that the **rigor mortis** which sets in when an animal dies is due to the clotting of muscle proteins.

Little is known about the functions of globulin X, myogen, and myoglobin of the muscle sarcoplasm. Myoglobin is a conjugated protein similar to hemoglobin. It contains iron, and its molecular weight is one-fourth that of the hemoglobin molecule.

Nonnitrogenous Extractives. The extractives of muscle may for convenience be divided into two classes, the **nonnitrogenous** and the **nitrogenous.** Of the nonnitrogenous type may be mentioned **glycogen, lactic acid, inositol,** and **lipids.** Glycogen is present in muscle in amounts ranging from 0.15 to 0.30 per cent. It constitutes the main source of the energy used in muscular contraction. Lactic acid is one of the products formed when sugar is metabolized by the muscle for energy. Inositol is an interesting compound, which may be looked upon as cyclohexane with an OH group attached to each carbon atom.



Inositol

The calicum-magnesium salt of inositol phosphoric acid, which is found in many plants, is called **phytin.** Phytin is not easily digested; hence many plant materials, although they may be rich in phosphoric acid according to their chemical analysis, are poor sources of phosphorus in the diet.

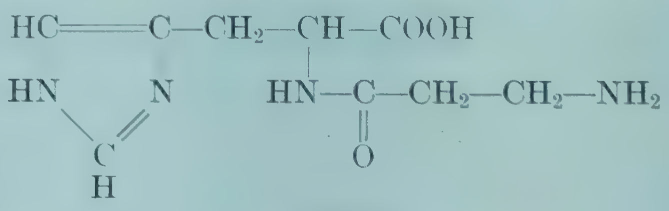
Inositol has been shown to be essential in the diet of mice, rats, and chicks for normal growth. When it is lacking in the diet, mice lose their hair, and rats develop a peculiar condition of the eye. Thus it appears that inositol is a vitamin.

Muscles contain lipids, which may be extracted with ether. In the muscle cells the lipid material is mainly phospholipid. Neutral fat, for the most part, is deposited between the muscle fibers in the connective tissue. Housewives are familiar with the fact that a steak which has a generous supply of fat distributed uniformly through the lean is a better steak than one which is all lean.

The inorganic salts of muscle are the sodium, potassium, iron, calcium, and magnesium salts of phosphoric and hydrochloric acids. Potassium phosphate is the predominating salt present.

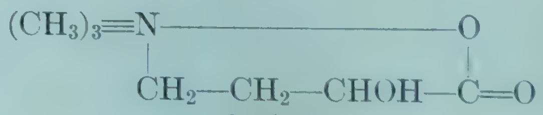
Nitrogenous Extractives. Among the important nitrogenous extractives obtained from muscle are **creatine, creatine phosphate, purines,** free and combined as **nucleotides, carnosine, anserine,** and **carnitine.**

Creatine and creatinine, as well as certain nucleotides such as adenylic acid and adenosine di- and triphosphates, play important roles in muscle metabolism, as was indicated in the discussion of carbohydrate metabolism. Carnosine is a dipeptide of histidine and β-alanine.



Carnosine

Anserine is methylcarosine, the methyl group replacing the hydrogen tied to nitrogen in the ring. Carnitine is a betaine.



Carnitine

The functions of carnosine, anserine, and carnitine are unknown.

A glance at the foregoing list of compounds found in the extractives of muscle tissue shows that little of nutritive value is present. It is evident that the broths which are often fed to invalids have but slight food value. Their main function in the diet appears to be their effect on the appetite. The value of soups in nutrition depends largely upon meat and vegetables present and not upon meat extractives.

Nervous Tissue. The nervous tissue has been called the master tissue of the body because by means of it all other tissues are controlled. Nervous tissue is found in the **brain, spinal cord,** and **nerves.** About 85 per cent of the nervous tissue is found in the brain. Most of our knowledge of the composition of nervous tissue has come from a study of the brain.

Structure of Nervous Tissue. Nervous tissue is made up of nerve cells called **neurons.** (See Fig. 25.) A typical neuron consists of a **cell body,** which contains a **nucleus.** From the surface of the cell body project several short branches, called **dendrites,** and a single long process, called an **axon.** At the end of the axon are **terminal branches.** The cell body, dendrites, and axon are the true nervous tissue through which the nerve impulses pass. This tissue is gray and is often called **gray matter.**

In most nerve cells the axon is covered with a fatty layer, the **myelin sheath,** which is white and is often called **white matter.** Surrounding the myelin sheath or, if this is absent, the axon itself, is a membrane called the **neurilemma.**

Nerve impulses pass from one nerve cell to another through the contact of the terminal branches of the axon of one cell with the dendrites of the cell body of another. These zones of contact are known as **synapses.** Nerves are attached to the muscle fibers or organs that they control by means of an **end organ.**

The myelin sheath, which is rich in lipids, is thought to serve as an insulating material for the axon. It has been suggested that it may also serve as a food supply for the nutrition of the nerve cell.

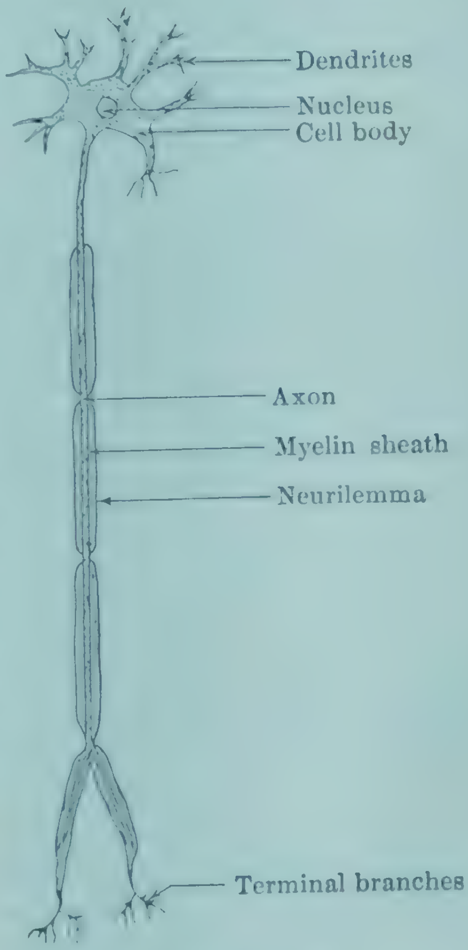


FIG. 25. A neuron.

Composition of Nervous Tissue. The most characteristic fact concerning the composition of the brain is its high content of **lipids.** Fresh brain contains from 12 to 15 per cent of lipid. There is very little, if any, neutral fat in the brain, the lipid fraction consisting of phospholipids, such as **lecithin, cephalin,** and **sphingomyelin;** glycolipids, such as **phrenosin, kerasin,** and **nervon;** and **cholesterol.**

From 8 to 9 per cent of fresh brain is protein. An albuminoid called **neurokeratin** has been isolated from brain; it differs in composition from other keratins. Several globulins are present, one of which coagulates at 47°C. It has been suggested that in sunstroke this globulin coagulates. Nucleoproteins which contain the usual animal nucleic acid are present.

Various extractives have been obtained from brain, among which the most important are creatine phosphate, adenosine triphosphate, inositol, and lactic acid.

Metabolism of Brain. The respiratory quotient of the brain has been found to be about 1, which indicates that carbohydrates are the main source of its energy. The main source of this carbohydrate is the glucose of the blood. When blood-sugar values become low weakness, mental confusion, and even convulsions may occur. This is the basis of the insulin-shock treatment for insanity. In this treatment the patient is given large doses of insulin which reduce the blood sugar to a point where mental confusion or even convulsions occur. After recovery from repeated treatments the mental condition of the patient is often greatly improved.

Glutamic acid appears to be the only amino acid metabolized in the brain. It is interesting that glutamic acid has been found helpful in the treatment of a mild form of epilepsy. It is also claimed to be of value when fed to mentally retarded children.

Nervous Control of Muscles. The stimulation of muscles has been explained on a chemical basis. Involuntary muscles, such as those found in blood vessels, are controlled by two types of nerves, **sympathetic** and **parasympathetic.** In the end organ of sympathetic nerves a compound similar to adrenaline called **sympathin** is produced which enters the muscle, causing it to contract. In the end organ of parasympathetic nerves **acetylcholine** is produced which enters the muscle causing it to relax. The actions of sympathin and acetylcholine are brief, because after they have served their purpose they are destroyed, the former by oxidation, and the latter by hydrolysis into choline and acetic acid. Sympathetic nerves are sometimes called **adrenergic** nerves because they produce an adrenaline-like substance; parasympathetic nerves are called **cholinergic** because they produce acetylcholine.

In voluntary muscles, which are controlled by the parasympathetic nervous system, acetylcholine causes contraction. Such muscles relax when acetylcholine is hydrolyzed.

Cerebrospinal Fluid. The brain and spinal cord are bathed in the cerebrospinal fluid, which contains about 99 per cent of water. Very little protein is present; its nonprotein constituents are about the same as those of blood. Normally from 3 to 5 white blood cells are present per cubic millimeter. In certain diseases the white-cell content increases, and bacteria may be present. The protein content may also increase. In meningitis the protein content and the white-cell count increase, and the specific organism causing the disease may be demonstrated to be present in the white cells. In the diagnosis of syphilis a Wassermann test is often made on the spinal fluid in preference to the blood.

Epithelial Tissue. The epithelial tissues, those which cover the outside of the body, include the outside layers of the skin, the nails, and the hair, and the horns, hoofs, and feathers of animals. The most important point with regard to their chemical composition is that they are composed largely of an albuminoid protein called **keratin**, which is characterized by its insolubility. It is not digested by gastric or pancreatic juice. It contains a high percentage of cystine. Human hair is very rich in cystine, containing from 16 to 21 per cent. Sheep's wool and feathers contain from 7 to 12 per cent of cystine.

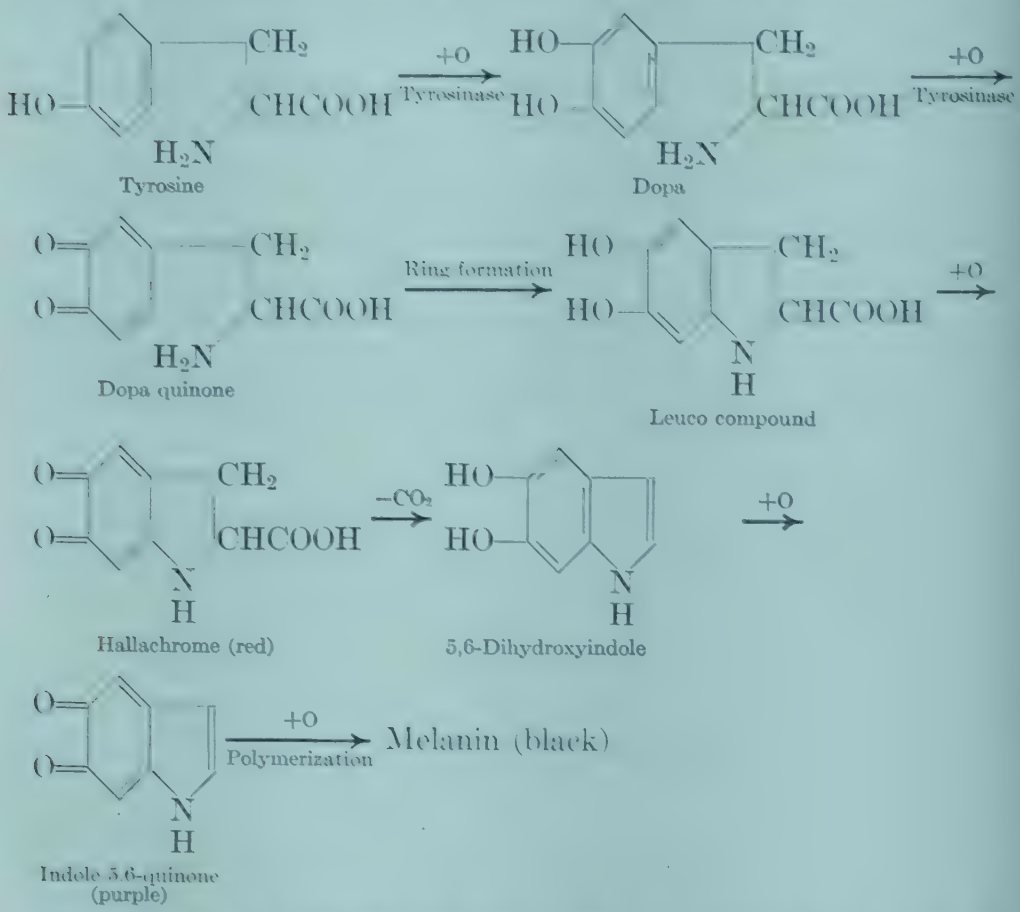
The keratin molecule is said to be made up of closely packed polypeptide chains held together by the S—S linkage of cystine, one-half of the cystine molecule being in one polypeptide chain and the other half in a neighboring chain. If keratins, such as hair, are ground to a fine powder, so that the S—S linkages between polypeptide chains are broken, they become more soluble and are digested by pepsin and trypsin. Clothes moths have an enzyme in their digestive tracts capable of digesting keratin.

When metallic poisons, such as arsenic, find their way into the body, they concentrate in the skin and hair. Cumulative arsenic poisoning, such as might occur from the long-continued use of vegetables or fruits sprayed with arsenicals, may be detected by the high arsenic content of the hair.

Pigmentation. The color of the skin, hair, and eyes is due to a pigment called **melanin,** from a Greek word *melas* meaning black. In the skin the melanin occurs in specialized cells called melanoblasts located in the basal layer of the epidermis. Much more melanin is present in the skin of a negro than in that of a white man. Melanin also accounts for the black hair and dark eyes of negroes. In white men the color of the hair and eyes varies depending on the amounts of melanin present.

In plants there are many examples of melanin formation among which may be mentioned the black color of certain mushrooms, the blackening of peeled potatoes, and the browning of sliced apples when exposed to the air.

Considerable knowledge has been gained concerning the chemistry of melanin formation. Melanin is derived from the amino acid **tyrosine** as a result of a series of reactions. In the presence of oxygen and the enzyme **tyrosinase**, tyrosine is first oxidized to 2,4-dihydroxyphenylalanine, usually referred to as "dopa," an abbreviation of the chemical name *di-oxy-phenyl-alanine*. Dopa is further oxidized by tyrosinase and oxygen to dopa-quinone. From here on the reaction does not require tyrosinase. Dopa quinone is converted into a leuco compound which oxidizes to a red compound called hallachrome. Hallachrome is next decarboxylated and rearranged to form 5,6-dihydroxyindole. This oxidizes to a purple compound, indole 5,6-quinone, which polymerizes with the addition of oxygen to form melanin. The chemical reactions involved in melanin formation may be summarized as follows:


CH₂
HO
CHCOOH
H₂N
Tyrosine
+O
Tyrosinase
HO
HO
CH₂
CHCOOH
H₂N
Dopa
+O
Tyrosinase
O
O
CH₂
CHCOOH
H₂N
Dopa quinone
Ring formation
HO
HO
CH₂
CHCOOH
N
H
Leuco compound
+O
O
O
CH₂
CHCOOH
N
H
Hallachrome (red)
−CO₂
HO
HO
N
H
5,6-Dihydroxyindole
+O
O
O
N
H
Indole 5,6-quinone
(purple)
+O
Polymerization
Melanin (black)

The purpose of pigment in the skin is to protect the individual against the harmful effects of the ultraviolet rays of the sun. Races that have developed in the tropics are highly pigmented, whereas those that have developed in the temperate zones are lighter in color. White men become protected against injury from the sun's rays by developing sun tans. In sun tanning the ultraviolet rays of the sun catalyze the oxidation of tyrosine to dopa by tyrosinase.

An interesting condition exists in albinos where there is no melanin in the skin, hair, or eyes, with the result that the skin and hair are white, and the eyes appear pink. Albinism is an inherited characteristic and is due to the absence of melanoblasts in the epidermis. Chemically it is attributed to the absence of tyrosinase which is located in the melanoblasts. Although adequate amounts of tyrosine are present in the epidermis, melanin cannot be formed from it in the absence of tyrosinase. Albinos do not sun tan and must avoid exposure to strong sunlight.

An interesting reaction occurs when normal skin is placed in a dilute solution of dopa at *p*H 7.35. In 24 hours melanin granules are deposited in the melanoblasts. Albino skin shows no response. This reaction is called the **dopa reaction.**

Connective Tissue. The connective tissues include the tissues of the **tendons** and **ligaments**, the **cartilages**, the **bones**, and the **teeth.** The organic matter of these tissues is largely protein of the albuminoid type. In the bones and teeth, mineral matter is deposited in an organic matrix.

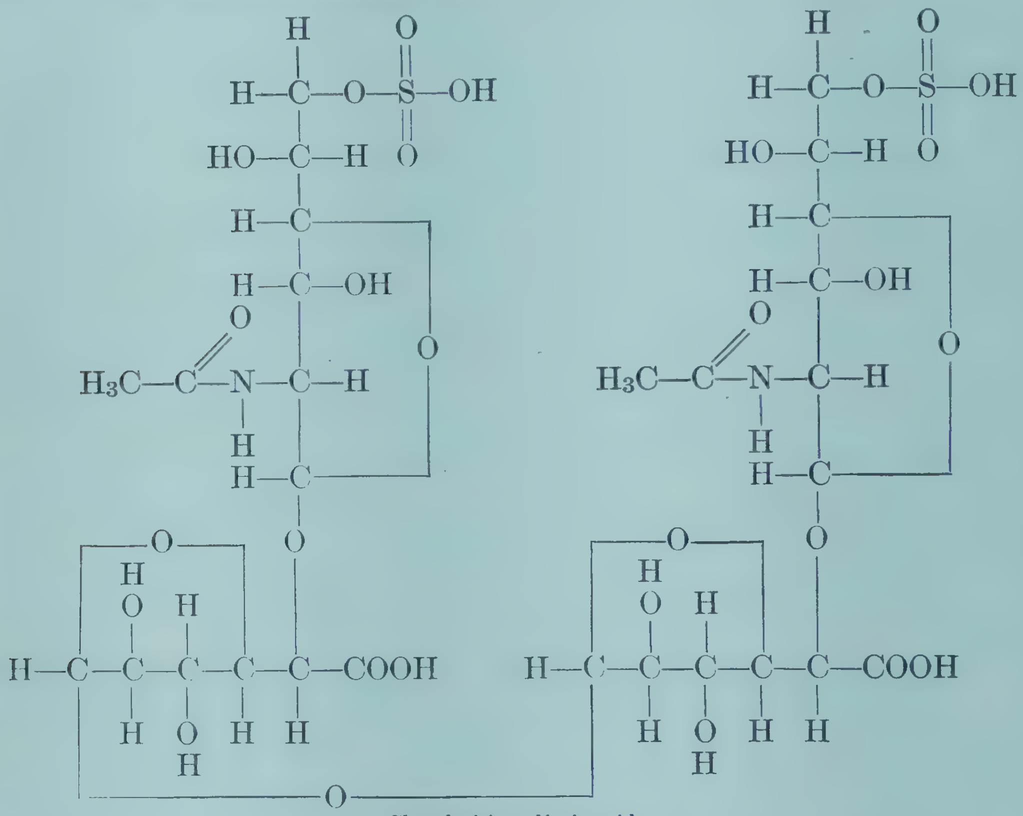
Tendons and Ligaments. Tendons and ligaments are composed of two types of connective tissue, namely, **white fibrous tissue** and **yellow elastic tissue.** The tendon of Achilles of the ox is a good example of white fibrous tissue. Its main constituent is an albuminoid called **collagen.** Collagen differs from the keratin of the epithelial tissue in that it is digested by pepsin and by trypsin after it has first been acted upon by pepsin. It also contains less sulfur than does keratin. On heating with water, it is converted into **gelatin.**

Gelatin is readily soluble in hot water and is easily digested. It is a common article in the diet. Gelatin is lacking in several amino acids and hence cannot supply the protein needs of the body. It is lacking in cystine, tryptophan, and tyrosine. However, it is especially rich in lysine and for this reason is a good supplement for cereal proteins which contain little lysine.

Among other proteins present are **elastin, tendomucoid,** and a small amount of coagulable protein. A small amount of lipid is also present.

Yellow elastic tissue is best represented by the *ligamentum nuchae* of the ox. It is characterized by its high content of the albuminoid **elastin.** It also contains considerable **collagen** and small amounts of the other substances found in white fibrous tissue. Like collagen, elastin is insoluble in water and is digested by the proteolytic enzymes of the digestive tract. It is not converted into gelatin when boiled with water.

Cartilage. Cartilaginous tissue is composed of **chondromucoid, chondroalbuminoid,** and **collagen.** Chondromucoid is a conjugated protein of the glycoprotein type. In composition it resembles the mucin of saliva. On partial hydrolysis it is broken down into protein and **chondroitin sulfuric acid.** On complete hydrolysis chondroitin sulfuric acid yields two molecules of **glucuronic acid,** two of **galactose amine,** two of **acetic acid,** and two of **sulfuric acid.** Various chondromucoids, which differ from one another in the nature of the protein present, exist.



Chondroitin sulfuric acid

A compound called mucoitin sulfuric acid, similar to chondroitin sulfuric acid in structure, is found in the mucin of the saliva. It differs from chondroitin sulfuric acid in that it contains acetylglucosamine in place of acetylgalactosamine.

If one removes the sulfuric acid from mucoïtin sulfuric acid, one gets **hyaluronic acid,** a compound composed of acetylglucosamine and glucuronic acid. Hyaluronic acid is a cementing material which holds cells together and serves as a lubricating material in joints. An enzyme called **hyaluronidase** is capable of hydrolyzing hyaluronic acid.

In arthritis, joint stiffness has been attributed to over-activity of this enzyme which destroys the lubricant in the joint. It may also hydrolyze hyaluronic acid present between the cells of the tissues surrounding the joints, thus allowing the fluid in the joint to escape. Hyaluronidase is present in certain pathogenic organisms, and their spread in tissues may be explained in part by their ability to destroy the hyaluronic acid present between the cells.

Chondroalbuminoid is similar to keratin and elastin. It is digested by pepsin.

Bone. Bone is composed of an organic matrix similar to cartilage in which large amounts of mineral matter are deposited. The organic matrix is composed of **osseomucoid**, **osseoalbuminoid**, and **collagen.** Because of the collagen present, bone serves as a good source of gelatin. Some bones are hollow and contain marrow which is rich in fat. There are two kinds of bone marrow, namely, yellow and red. Red bone marrow is one of the main tissues involved in red-blood-cell formation.

The mineral matter of dried, fat-free bone comprises about 60 per cent of its weight. The composition of the mineral matter in bone may be represented by the formula: $3Ca_3(PO_4)_2 \cdot CaX_2$, where X_2 is usually CO_3 but may be F_2, SO_4, O, or $(OH)_2$. On the percentage basis the ash of bone contains about 85 per cent of $Ca_3(PO_4)_2$, 10 per cent of $CaCO_3$, and 1.5 per cent of $Mg_3(PO_4)_2$.

The deposition of calcium and phosphorus in bone is thought to involve the activity of the enzyme **phosphatase.** This enzyme is known to be present in bone, and it is thought that it hydrolyzes organic phosphates, such as hexose phosphates, liberating phosphoric acid. The phosphate ion then precipitates as $Ca_3(PO_4)_2$, provided that the proper concentrations of Ca^{++} and PO_4^{---} are present and also that the pH is favorable.

That the minerals of bone are not in an inert form has been shown by feeding radioactive phosphorus compounds. In a short time radioactive phosphorus is found deposited in the bones.

Calcification of bone has been studied in connection with the disease known as rickets, which is characterized by a poor deposition of calcium and phosphorus. Factors which influence calcification are the amounts and ratios of calcium and phosphorus in the diet, and vitamin D or sunlight. Vitamin D is essential for the proper absorption and utilization of calcium and phosphorus. Vitamins A and C are also important for proper bone formation.

Hormones of the parathyroid and pituitary glands have an influence on bones. If the parathyroid glands are overactive, the bones are decalcified and weakened, and blood-calcium values rise. A hormone of the pituitary gland regulates bone growth. If this hormone is overproduced in a young person, the long bones overgrow and gigantism results. In the case of an older person, the bones of the skull become misshapen (acromegaly). If the hormone is underproduced in a young person, dwarfism results.

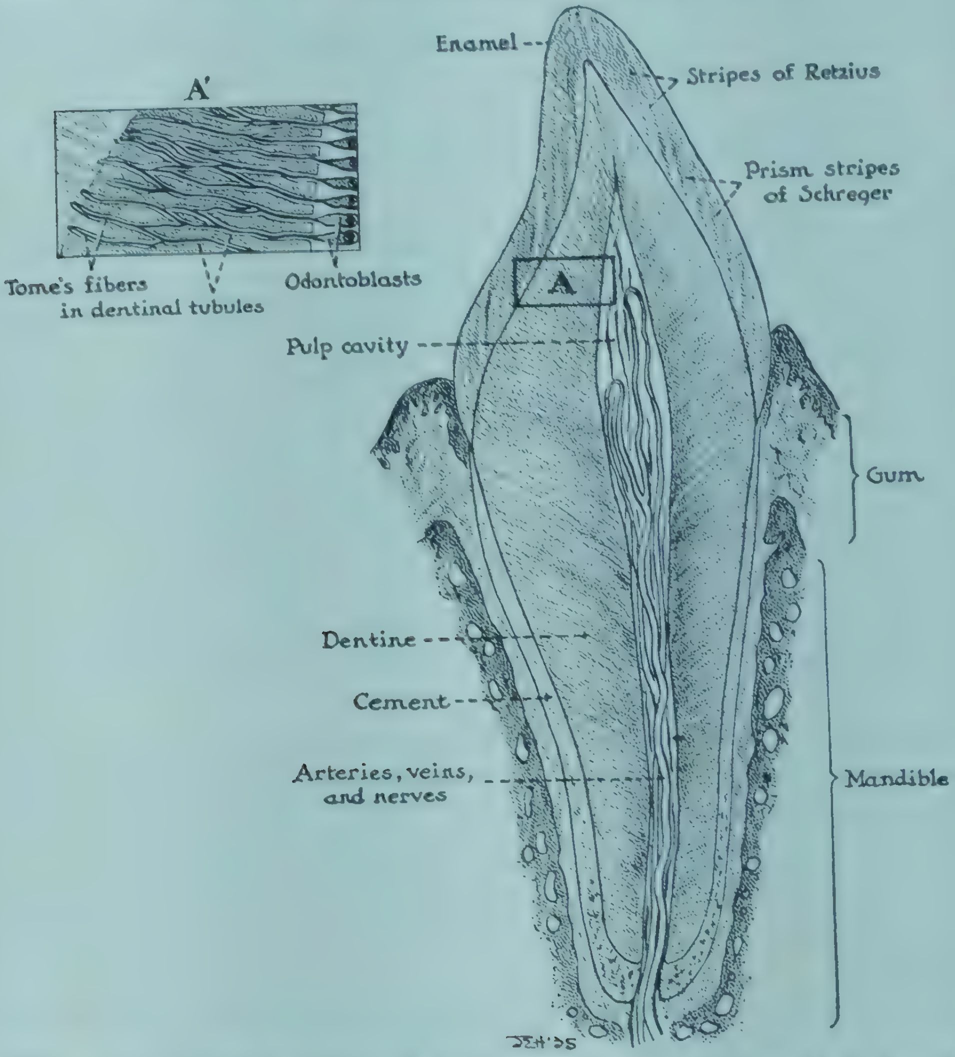

Enamel
Stripes of Retzius
A′
Prism stripes of Schreger
Tome's fibers in dentinal tubules
Odontoblasts
A
Pulp cavity
Gum
Dentine
Cement
Arteries, veins, and nerves
Mandible

Fig. 26. Longitudinal section of an incisor tooth. The part *A* is shown at high magnification in *A′*. From *Physiological Chemistry* by J. F. McClendon and C. J. V. Pettibone. Courtesy of C. V. Mosby Co.

Teeth. The bony structure of teeth consists of three parts, namely, **enamel, cement,** and **dentine.** Through the center of each root of a tooth there is a narrow canal filled with pulp tissue, embedded in which are the nerve and blood vessels. (See Fig. 26.) The enamel, cement, and dentine are bony structures consisting of an organic matrix in which mineral matter is deposited.

Enamel is the material that covers the exposed surface of teeth. It is the hardest substance in the body and contains about 96 per cent of mineral matter consisting mainly of $3Ca(PO_4)_2 \cdot Ca(OH)_2$. The organic matrix consists mainly of **keratin** and in this respect resembles epithelial tissue.

Cement is a material very similar to bone in composition, which forms a layer covering the roots of the teeth. It contains about 50 per cent of mineral matter. The organic matrix consists largely of **collagen** and thus resembles white connective tissue in composition.

The main body of a tooth is composed of dentine which contains about 70 per cent of mineral matter. The organic matrix of dentine is composed mainly of **collagen,** with some elastin present.

Metabolism of Teeth. The mineral metabolism of teeth is much less active than that of bones. When radioactive phosphates are fed they do appear in the teeth but at a much slower rate than in the bones. Very little if any appears in the enamel of mature teeth; most of that which does appear is in the dentine. Thus teeth do not act as a reserve mineral supply as do bones. At one time it was thought that during pregnancy mineral matter was withdrawn from the teeth, resulting in decay, but this is not the case. Teeth of pregnant and nonpregnant women have been found to contain the same amount of ash. Pregnancy is not a factor in tooth decay.

DENTAL CARIES. This is a term applied to tooth decay. Much work has been done to determine the cause of dental caries, but as yet no satisfactory explanation of its etiology has been found. It is likely that many factors are involved in this condition. Best information at the present time indicates that for sound teeth there must be an adequate supply of absorbable calcium and phosphorus in the diet. In addition, the diet should furnish sufficient vitamins, especially vitamins A, C, and D.

If vitamin A is inadequate in childhood, there is said to be a greater susceptibility to dental caries in later life. Lack of vitamin A brings about changes in the pulp tissue of the teeth and also alters the mineral matter, especially by increasing the magnesium content of the ash. Vitamin C deficiency causes hemorrhage and degeneration of the pulp tissue and also imperfect calcification. In addition, the gums may bleed and degenerate, resulting in a loosening of the teeth. Lack of vitamin D results in poor calcification.

Another factor which appears to be related to dental caries is the amount of fluorine in the diet. It has been noted that in certain regions of Arizona where there is much fluorine in the drinking water there is very little dental caries. In the eastern states, where there is little fluorine in the water, dental caries is common. Fluorine contributes hardness to the enamel of teeth and, along with hardness, resistance to decay.

Many cities are now adding fluorides to their water supply to lower the incidence of dental caries. Where this has been done for many years dental caries has been reduced. Fluorides may be applied to the surface of teeth with beneficial results. This should be done by a dentist since fluorides are very toxic and must be used with care. The treatment is only used for children while teeth are in their formative stages; it is of little value in adults. It has been said that this treatment lowers the incidence of dental caries 40 per cent.

Some believe that tooth decay is due primarily to bacterial action. After eating, food deposits between the teeth and in crevices in the enamel. Bacteria act on these food deposits, producing acids which dissolve the mineral matter of the teeth and thus provide an entrance for bacteria to continue the destruction. If this is true, the brushing of teeth after each meal should be a good preventive of dental caries.

It is commonly believed that eating candy causes tooth decay. Candy may contribute to tooth decay in two ways. First, sugar is easily fermented by bacteria to acids which may dissolve the minerals of the teeth; second, a child eating much candy satisfies his appetite and does not eat sufficient food containing the proper amounts of minerals and vitamins for the formation of sound teeth.

Many persons go through life without having dental caries. It would appear that they either have inherited sound teeth or have had the proper diet during the period when their teeth were being formed. It is said that the Eskimo of Alaska had excellent teeth before the advent of the white man, but now that he is eating our food, he has as much dental caries as we do.

There is little doubt that the ultimate decay of teeth is due to bacteria, but whether the fundamental cause is bacterial action is questionable. It appears that the fundamental cause of dental caries is a deep-seated physiological disturbance, perhaps related to diet. If the diet lacks the proper vitamins and the proper materials for building teeth, tooth structure is impaired, a situation which makes possible the destruction of the teeth by bacterial action.

REVIEW QUESTIONS

1. Name four proteins found in muscle. Which protein is associated with muscular contraction. What is actomyosin?
2. What happens to muscle protein during rigor mortis?
3. Name the nonnitrogenous extractives of muscle.

4. What is phytin? What evidence is there that inositol is a vitamin?
5. Name the nitrogenous extractives of muscle.
6. Discuss the nutritive value of broth.
7. Name three parts of the body that are composed of nervous tissue.
8. Draw a diagram of a nerve cell and label all parts.
9. Name several compounds found in nervous tissue. To what group of compounds do most of them belong?
10. What is the main source of energy for the brain?
11. What amino acid is metabolized in the brain?
12. Give a chemical explanation of how the contraction and relaxation of involuntary muscles are controlled.
13. Discuss the cerebrospinal fluid.
14. What is the main protein in epithelial tissue?
15. Give examples of melanin pigmentation in animals and plants. From what is melanin formed? Give the chemical reactions in its formation.
16. How may albinism be explained on a chemical basis?
17. What is meant by the "dopa" reaction?
18. Name the tissues included in the connective tissue.
19. Name the proteins found in white fibrous tissue and in yellow connective tissue.
20. How is gelatin prepared?
21. Name the proteins found in cartilage.
22. Into what simple molecules does chondroitin sulfuric acid break down on complete hydrolysis?
23. What are hyaluronic acid and hyaluronidase? How may they be related to joint diseases?
24. Discuss the structure and composition of bone.
25. Name three vitamins and two hormone-producing glands associated with bone formation.
26. Discuss the relation of phosphatase to bone calcification.
27. Of what four parts are teeth composed?
28. Is the mineral metabolism of teeth as active as that of bones?
29. Discuss the causes and prevention of dental caries.

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17

Blood

The blood has been called the **circulating tissue** of the body. It is perhaps difficult to think of blood as a tissue, but in view of the fact that in a cubic millimeter of normal blood there are in the neighborhood of 5,000,000 cells, this designation seems not unreasonable. There are about 6 quarts of blood in a man of average size. Briefly, the functions of the blood are: (1) to convey food materials to the body cells and to remove waste products from them; (2) to carry the oxygen used in the oxidation of foods and to remove carbon dioxide resulting from such oxidation; (3) to transport the internal secretions and vitamins which control the activities of the body; (4) to aid in the control of pH, water content, and temperature of the tissues; and (5) to assist the body's defense against bacterial disease by means of antitoxins or by leucocytes which have the ability to destroy bacteria.

Lymph. The blood itself does not come into direct contact with the cells of the body. The body cells and also the blood vessels are bathed in a liquid called **lymph.** The transfer of materials to and from the blood and body cells takes place through the lymph, which resembles quite closely the liquid part of the blood, called the **plasma.** The main difference between lymph and blood plasma is that the former contains less protein then the latter.

Lymph contains some white blood corpuscles called **lymphocytes.** It circulates to some extent through a system of vessels called the **lymphatics.** In its circulation it passes through lymph glands and finally enters the blood stream near the junction of the jugular and the left subclavian veins under the left shoulder. As the blood circulates to the tissues, lymph is secreted from it, and thus the circulation of the lymph is completed.

Constitution of Blood. Blood is made up of **formed elements** suspended in a liquid called **plasma.** The formed elements are the red blood cells or **erythrocytes,** the white blood cells or **leucocytes,** and the blood **platelets.** If blood to which an oxalate has been added to prevent clotting is allowed to stand, the formed elements settle out, leaving the plasma, a straw-colored liquid, at the top.

About 55 per cent of the volume of blood is plasma, and about 92 per cent of plasma is water. Of the 8 per cent solids in plasma, about 7 per cent is protein. The protein is composed of albumin, α-, β-, and γ-globulins and euglobulin, and fibrinogen. About 1 per cent of plasma is made up of mineral salts composed of such elements as sodium, potassium, calcium, phosphorus, and chlorine. Also present are amino acids, lipids, glucose, and nitrogenous waste products of metabolism, such as urea, uric acid, creatine, and creatinine.

Formed Elements. Normal blood of men contains about 5,000,000 red cells per cubic millimeter. The normal value for women is somewhat lower, usually being given as 4,500,000. The red cells contain no nuclei and are described as biconcave discs. The red color of these cells is due to the presence of a very important pigment called **hemoglobin,** which carries oxygen from the lungs to the tissues. The red blood cells are composed of a protoplasmic tissue called the **stroma,** in which the hemoglobin is held. Various reagents, such as water, ether, and soap, when added to blood cause disintegration of the red cells, the hemoglobin being liberated, so that a clear red solution is formed. Blood in which the red cells are destroyed is said to be **hemolyzed** or **laked.** Unhemolyzed blood is opaque.

The white blood cells are present in normal blood to the extent of 5000 to 10,000 per cubic millimeter. There are several varieties of white cells, most of which are larger than red cells. All contain nuclei. One of the main functions of white blood cells is to aid the body's defense against disease. Bacteria become engulfed in white blood cells, and they are destroyed by a process known as **phagocytosis.**

Blood platelets are normally present in blood to the extent of about 350,000 per cubic millimeter. They are much smaller than red cells and are irregular in shape. They contain no nuclei and in a stained slide usually appear in groups. Blood platelets are an important factor in blood clotting. Figure 28 shows the appearance of the formed elements of normal blood under the microscope.

Blood Counting. *Red Cells.* One of the most common tests in a clinical laboratory is the determination of the number of the various formed elements present in blood. The reason for this procedure is that there is a wide fluctuation from normal in disease. In anemia red-cell counts are low, and in many infections white-cell counts are high.

The number of red blood cells per cubic millimeter of blood is determined by an actual count under the high power of a microscope. By means of an accurate pipette a small volume of blood is measured and diluted 200 times with a special diluting fluid. A uniform suspension of the cells is obtained by shaking, and a drop of the suspension is placed on a counting chamber and covered with a cover slip. The rulings on the counting chamber cover an area of 9 sq. mm. (See Fig. 27.) The central square millimeter is ruled off into 400 small squares, which are used in making a red-cell count. The counting chamber has a depth of 0.1 mm. The number of cells in 80 small squares multiplied by 10,000 gives the number of red cells per cubic millimeter of the original blood.

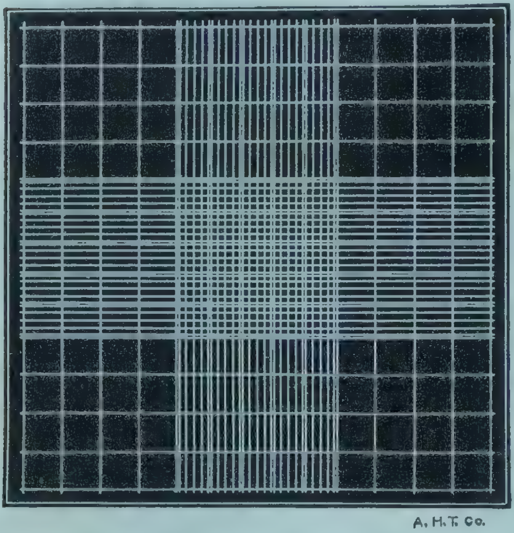


FIG. 27. Improved Neubauer ruling on a blood-counting chamber.

White Cells. The white blood cells or leucocytes are counted in much the same manner as the red cells. The diluting fluid is 1.5 per cent acetic acid, which destroys the red cells and makes the white cells more easily seen. Because of the smaller number the dilution is 1–20 instead of 1–200, as for a red-cell count. The count is made under the low power of the microscope, and an area of 1 sq. mm. is the unit counted. The number of cells per square millimeter multiplied by 200 gives the number of cells per cubic millimeter of the original blood.

The function of white blood cells in the body is to combat infections. When there is an acute infection in the body, the number of white blood cells usually increases. A leucocyte count aids a physician in the diagnosis of such diseases as appendicitis. In acute appendicitis the leucocyte count often reaches 15,000 to 20,000.

Other diseases also affect the leucocyte count. Often higher leucocyte counts are found in pneumonia than in appendicitis. High counts also accompany ear infections. In fact, many infections are accompanied by high leucocyte counts. The disease in which the leucocyte count increases to the greatest extent is leukemia, in which counts of 200,000 to 500,000 are found. An increase in the number of leucocytes in the blood is often referred to as **leucocytosis.**

In addition to leucocytosis there are also conditions where the leucocyte count is below normal. This condition is often referred to as **leucopenia.** Leucopenia often occurs after heavy doses of x-rays.

Differential Count. The leucocytes are not all of the same type. In normal blood it is easy to distinguish seven different kinds of leucocytes. (See Fig. 28.) It is of great diagnostic value to a physician to know not only the total leucocyte count but also the proportion of the various types of leucocytes present. To determine the types of leucocytes in blood a **differential count** is made. This procedure involves spreading a drop of blood in a thin film on a microscope slide. This film is then stained with **Wright's stain,** which contains eosin, a red dye, and methylene blue, a blue one. The stained slide is then examined under the microscope, using the oil-immersion objective. During this examination every leucocyte is counted and classified, and after several hundred cells have been counted the percentage of each type present is determined. The following list names the various types of leucocytes and gives the percentage of each found in normal blood.

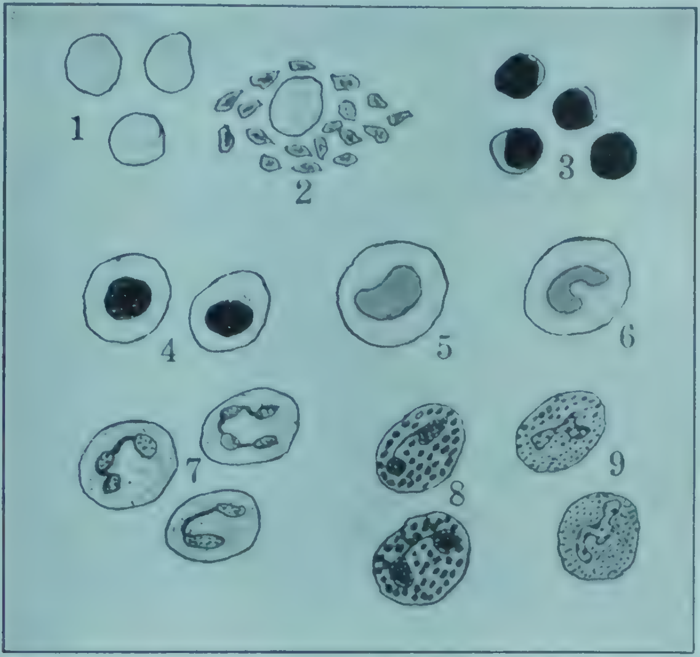


FIG. 28. Cells found in normal blood: (1) erythrocytes; (2) erythrocyte surrounded by blood platelets; (3) small lymphocytes; (4) large lymphocytes; (5) large mononuclear; (6) transitional; (7) polymorphonuclears; (8) eosinophiles — the stippling is red; (9) basophiles or mast cells — the stippling is blue.

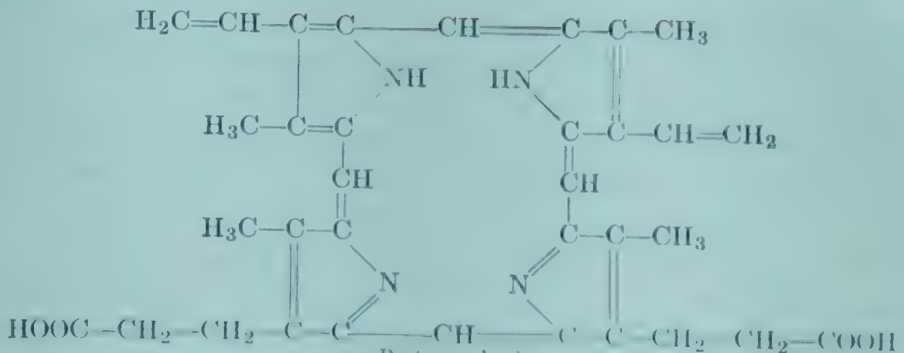
	PER CENT
Polymorphonuclears	65–70
Small lymphocytes	20–30
Large lymphocytes	2–6
Large mononuclears	1–2
Transitionals	2–4
Eosinophiles	1–2
Basophiles	0.25–0.5

The leucocytes are larger than the erythrocytes, and all have nuclei. The polymorphonuclears are easily distinguished because the nucleus is very irregular in shape. The lymphocytes have a very large round nucleus which almost fills the cell and stains dark blue. The small and large lymphocytes differ only in their size. The large mononuclears are larger than the large lymphocytes and stain a lighter blue. Transitionals are characterized by a kidney-shaped nucleus. Eosinophiles have somewhat the appearance of polymorphonuclears but are stippled a deep red. In other words, they take the eosin of the Wright's stain. Basophiles are somewhat similar to eosinophiles, except that they are stippled a deep blue.

In disease the proportion of the various types of leucocytes varies from the normal, and also other types of leucocytes appear. In appendicitis the percentage of polymorphonuclears may be from 80 to 90 or even higher. A high leucocyte count, together with 85 to 90 per cent of polymorphonuclears, indicates the need for an immediate operation in a case of appendicitis. In lymphatic leukemia the large increase in leucocytes is due to an increase in the number of lymphocytes. Thus the polymorphonuclear count is very low, and the lymphocyte count proportionately high. In infectious mononucleosis the percentage of large mononuclears increases. Intestinal parasites often cause an increase in the percentage of eosinophiles. High eosinophile counts are also encountered in rheumatoid arthritis. Eosinophile counts are used to differentiate between rheumatoid and other types of arthritis.

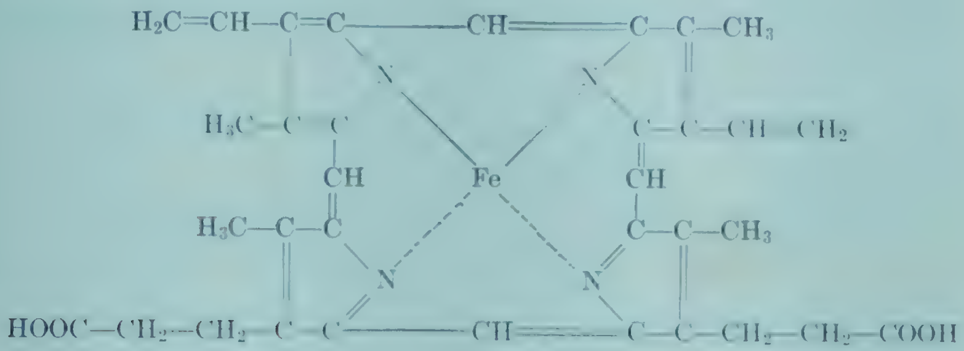
Blood Platelets. In making a platelet count a stained preparation is used. Red cells and platelets are counted, and their relative numbers are determined. If the red-cell count is known, it is then easy to estimate the platelet count. The blood platelets, as will be pointed out later, are important in the clotting of blood. Platelet counts are high in Hodgkin's disease and low in **purpura hemorrhagica,** a disease in which there is bleeding from the mucous membranes and under the skin, with the formation of black-and-blue spots.

Hemoglobin. Before leaving the subject of the formed elements of blood, it is necessary to consider hemoglobin, the pigment which gives the red blood cells their color. Hemoglobin is important because it acts as a carrier of oxygen from the lungs to the tissues. It is a conjugated protein made up of **heme,** the red pigment, and **globin,** a protein. It is interesting to note that heme is very closely related to chlorophyll, the bluish green pigment in plants. The main difference is that heme contains iron, whereas chlorophyll contains magnesium. Both heme and chlorophyll are made up of nitrogen-containing rings called **pyrrol** rings. The pyrrol complex which is the mother substance of heme is **protoporphyrin,** which has the following formula:



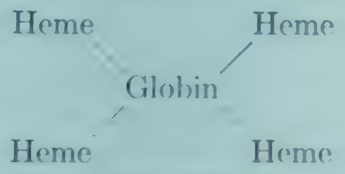
Protoporphyrin

Heme differs from protoporphyrin in that it contains an atom of ferrous iron.



Heme

Hemoglobin is a combination of one molecule globin and four molecules of heme, the globin being attached to heme through iron.



Hemoglobin

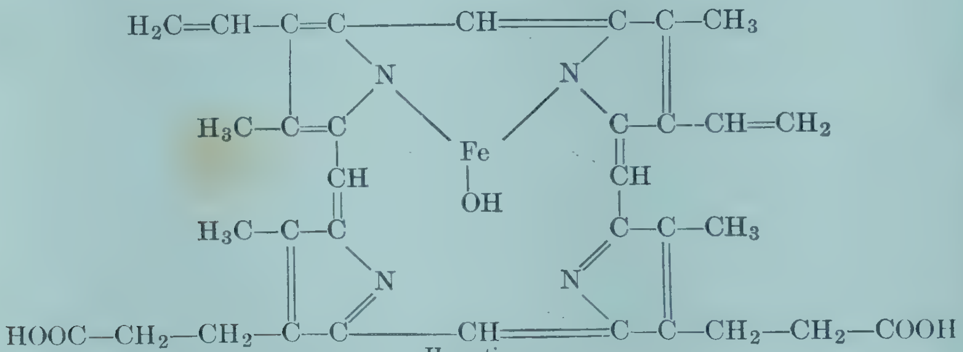
In certain animals, such as crabs and molluses, a protein-pigment complex containing copper is found in the blood. This pigment is called **hemocyanin** and is blue. Manganese has been shown to be the metal in the respiratory pigment of the tropical mussel, which in the oxidized form is brown.

Oxyhemoglobin. Hemoglobin that has not been exposed to air is purple in color. When exposed to air, it takes up a molecule of oxygen, forming oxyhemoglobin, which is scarlet in color. This change can be readily observed in a meat market. When freshly cut, meat is purple on account of the reduced form of hemoglobin inside the tissue. Shortly after cutting, it takes on a scarlet color due to oxidation by the air.

The iron in oxyhemoglobin remains ferrous, a molecule of oxygen being attached loosely to each of the four iron atoms of hemoglobin. The formula for oxyhemoglobin may be represented as globin (heme-O_2)$_4$. Usually the formula for oxyhemoglobin is given as HbO_2. Oxyhemoglobin is a rather unstable compound and readily gives up its oxygen to the tissues, forming hemoglobin again. This is what happens as the blood circulates through the body.

Methemoglobin. When oxyhemoglobin is treated with potassium ferricyanide it loses its oxygen and its iron is oxidized to the ferric state, forming methemoglobin which is brown in color. The brown color of meat under certain conditions may be accounted for by the presence of methemoglobin.

The iron-porphyrin complex in methemoglobin is called **hematin** and has the following formula:



Hematin

Carboxyhemoglobin. If oxyhemoglobin is treated with carbon monoxide, the carbon monoxide will replace the oxygen, forming carboxyhemoglobin, which is bluish red in color and is much more stable than oxyhemoglobin. In carbon monoxide poisoning the hemoglobin of the blood combines with the carbon monoxide and thus is no longer able to carry oxygen for respiration purposes. Carbon monoxide is an extremely dangerous substance because it has no odor and its action is rapid. Extremely small traces of carbon monoxide in the air gradually accumulate in the blood and finally cause death. When the blood becomes 60 per cent saturated with carbon monoxide death follows. Some of the symptoms of carbon monoxide poisoning are headache, dizziness, dim vision, nausea, unconsciousness, and convulsions. A poisoned person should be given artificial respiration in fresh air and a blood transfusion, if necessary.

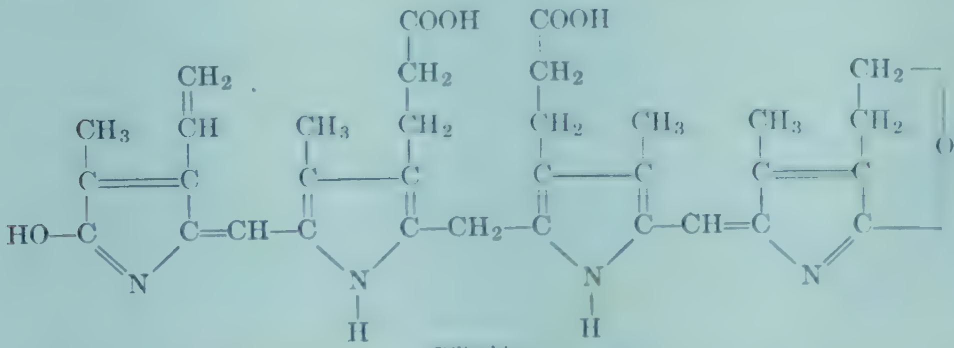
Hemin. If blood is treated with sodium chloride and acetic acid and heated gently, brown crystals of hemin are formed. In this reaction globin is removed from heme, and the iron is oxidized to the ferric state.

Hemin is the hydrochloride of ferric heme. The formula for hemin is the same as that for hematin, except that the hydroxyl attached to iron is replaced by chlorine.

Hemochromogen. If hemoglobin is treated with alkali in the presence of a reducing agent, the result is a pink pigment called hemochromogen, which differs from hemoglobin in that the globin is denatured. Many synthetic hemochromogens have been prepared by combining heme with various nitrogenous compounds such as nicotine, ammonia, pyridine, or albumin. A hemochromogen may therefore be defined as a combination of heme and some nitrogenous substance.

Bile Pigments. It will be recalled that in Chapter 10 on intestinal digestion the relationship of bile pigments to hemoglobin was discussed. (See p. 223.) Now that we have an understanding of the chemical structure of hemoglobin, we shall consider the origin of the bile pigments from it. The first step in the conversion of hemoglobin to biliverdin, the original bile pigment, is the opening of the porphyrin ring of heme and the formation of a compound called **choleglobin.** Next choleglobin loses its iron, forming a **globin-biliverdin complex.** Finally, this complex breaks down forming **biliverdin.** Biliverdin on reduction gives **bilirubin,** the main bile pigment in man.

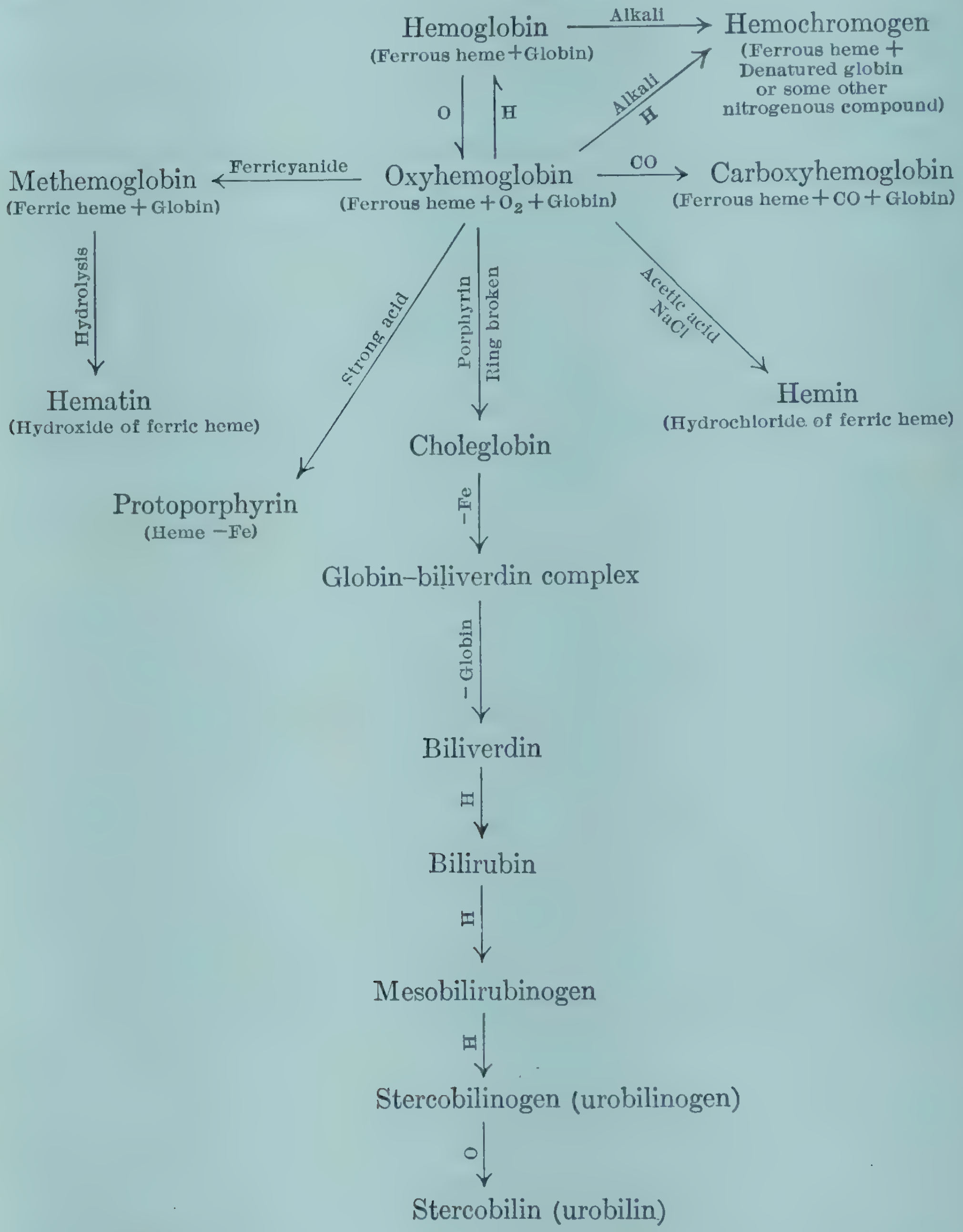
In the intestine bilirubin is reduced to **mesobilirubinogen,** which on further reduction, gives **stercobilinogen.** This on oxidation gives **stercobilin,** the brown pigment of the feces. Some stercobilinogen and stercobilin are reabsorbed and excreted in the urine, giving it a yellow color. In the urine these pigments are called **urobilinogen** and **urobilin,** respectively.



Bilirubin

The relationships existing between hemoglobin and its various derivatives are indicated in the diagram on p. 320.

Determination of Hemoglobin. The amount of hemoglobin in blood may be determined by the **Newcomer method,** in which a known volume of blood is diluted with a known volume of 0.1 N HCl. The hemoglobin changes to acid hematin, which is brown in color, the intensity of the brown color being proportional to the amount of hemoglobin present. By comparing the color produced with a standard color in a colorimeter, the amount of hemoglobin in the original blood may be calculated. According to Haldane, normal blood contains 13.8 grams of hemoglobin per 100 ml. It is customary to report hemoglobin values as percentages of normal. Thus, if a person has 6.9 grams of hemoglobin per 100 ml. of blood, he has 50 per cent normal hemoglobin. Since the various authorities do not agree on the percentage of hemo-


Hemoglobin
(Ferrous heme + Globin)
Alkali
Hemochromogen
(Ferrous heme + Denatured globin or some other nitrogenous compound)
O
H
Alkali
H
Methemoglobin
(Ferric heme + Globin)
Ferricyanide
Oxyhemoglobin
(Ferrous heme + O2 + Globin)
CO
Carboxyhemoglobin
(Ferrous heme + CO + Globin)
Hydrolysis
Hematin
(Hydroxide of ferric heme)
Strong acid
Porphyrin Ring broken
Acetic acid NaCl
Hemin
(Hydrochloride of ferric heme)
Choleglobin
Protoporphyrin
(Heme −Fe)
−Fe
Globin–biliverdin complex
−Globin
Biliverdin
H
Bilirubin
H
Mesobilirubinogen
H
Stercobilinogen (urobilinogen)
O
Stercobilin (urobilin)

globin in normal blood, it would be well if laboratories reported actual hemoglobin values rather than percentage of normal values. If this were done, values obtained in different laboratories would be comparable. Sahli believes that normal blood contains 17.2 grams of hemoglobin per 100 ml. Thus, if Sahli's method is used for determining hemoglobin, a sample containing 6.9 grams of hemoglobin per 100 ml. will be 40.1 per cent normal. It is apparent, then, that Sahli's method gives results about 20 per cent lower than the method of Haldane.

In the author's laboratory a study of the normal values for hemoglobin revealed that for one hundred healthy college men the average grams of hemoglobin per 100 ml. of blood was 14.96; for eighty-four healthy women, it was 13.16. These figures mean that, taking Haldane's value of 13.8 as normal, men should have about 110 per cent and women about 90 per cent normal hemoglobin values.

These are the grams of hemoglobin per 100 ml. of normal blood, according to the various workers in this field:

	GRAMS
Dare	13.77
Haldane	13.80
Newcomer (Williamson)	16.92
Oliver	15.00
Sahli	17.20
Tallquist	15.80
Von Fleischl-Miescher	15.80

Anemia. Red-cell counts and hemoglobin determinations are of great value to a physician in diagnosing anemia. Anemia is a condition in which the number of red cells or the percentage of hemoglobin or both are below normal. If the percentage of hemoglobin is reduced more than the cell count, the blood has a lighter color than the red-cell count would indicate. Such an anemia is called a **chlorotic anemia.** The term chlorotic is derived from a condition in plants called chlorosis, in which the leaves are light colored because of a lack of chlorophyll. In chlorotic anemia the color index is low. By **color index** is meant the percentage of normal hemoglobin divided by the percentage of normal red cells, 5,000,000 red cells per cubic millimeter being considered normal. Normally the color index should be 1. In chlorotic anemia the per cent of normal hemoglobin may be 60 and the red-cell count 4,000,000, or 80 per cent of normal. The color index will then be

$$\frac{\text{Per cent normal Hb}}{\text{Per cent normal red cells}} = \frac{60}{80} = 0.75$$

Chlorotic anemias indicate trouble with hemoglobin synthesis rather than with red-cell formation. In some types of anemia the color index is high, indicating a lack of ability to produce red cells rather than an inability to synthesize hemoglobin. Anemias may be divided into two classes, namely, **primary,** or **pernicious anemia,** and **secondary anemia.**

Primary Anemia. A primary anemia, usually spoken of as **pernicious anemia,** is an anemia in which the activity of the blood-building tissue has been decreased. Red blood cells, and also the white, originate in the reticulo-endothelial system lining the blood vessels of the liver, spleen, and bone marrow. The average life of a red blood cell is 4 months. Normally red cells are being destroyed as fast as they are being formed. If red cells are not being produced as fast as they are being destroyed, an anemic condition results. In pernicious anemia both the red-cell count and the percentage of hemoglobin are reduced. However, the percentage of hemoglobin is higher than would be expected from the count. In other words, there is a high color index. Counts of less than 1,000,000 red blood cells per cubic millimeter have been found in severe pernicious anemia. An early symptom of this disease is a lemon-yellow complexion, caused by the pigment arising from the destruction of red cells. In the laboratory pernicious anemia can be distinguished from secondary anemia by the microscopic appearance of the red blood cells. (See Fig. 29.) In pernicious anemia they are abnormal in shape; some of them are much larger than normal, and others may have an irregular form. Other conditions which accompany pernicious anemia are a lack of hydrochloric acid in the gastric juice and the appearance of a very red tongue.

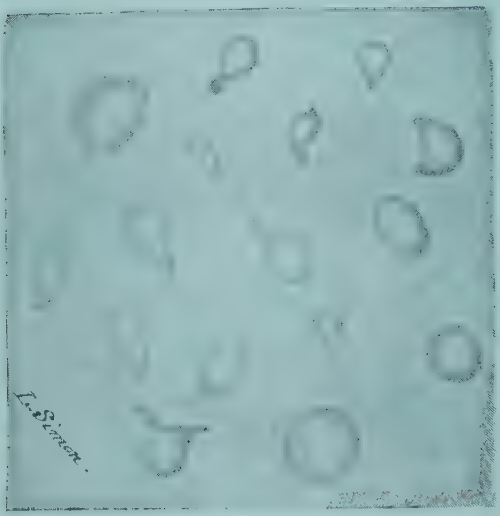


FIG. 29. Variation in shape and size of red blood cells in pernicious anemia. From *Clinical Diagnosis* by Simon. Courtesy of Lea & Febiger.

At one time pernicious anemia was considered a fatal disease. Now it is known that the inability of the bone marrow to make red cells is due to a lack of some stimulatory substance. This stimulatory substance is found in liver. Minot and Murphy have shown that pernicious anemia can be controlled by eating large quantities of liver or by taking a specially prepared liver extract. More recently it has been shown that an extract of stomach tissue will stimulate blood production. A combination of liver and stomach extract seems to be more efficient than either extract alone. From the fact that hydrochloric acid is lacking in the gastric juice of patients suffering from pernicious anemia it appears probable that a gastric disturbance is an important factor in the etiology of this disease.

Castle has reported that pernicious anemia patients show improvement when fed beef muscle which has been digested with the gastric juice of a normal person at a *p*H of 5 to 7. Neither normal gastric juice nor beef muscle digested with pure pepsin or gastric juice from pernicious anemia patients produced any effect when fed. From this fact Castle concluded that two factors were necessary to stimulate blood production. The one present in beef muscle he called the **extrinsic factor,** and the other, present in normal gastric juice, he called the **intrinsic factor.** Of the nature of these two factors little is known. There is some evidence to indicate that the intrinsic factor may be a proteolytic enzyme other than pepsin or trypsin.

Latest work on the treatment of pernicious anemia indicates that certain vitamins of the B complex, namely, folic acid and vitamin B_{12}, are helpful. Folic acid is effective in the treatment of the anemias of pellagra, sprue, and pregnancy, but will not relieve the nervous symptoms of pernicious anemia. It is therefore not the anti-pernicious anemia factor. Vitamin B_{12} is better in that it controls the nervous symptoms. It has been suggested that both folic acid and vitamin B_{12} are necessary and that they function in the following manner. Folic acid is necessary for the synthesis of thymine which is essential for the synthesis of a nucleoside called thymidine. Thymidine is necessary for the synthesis of nucleic acid needed for the production of red blood cells. In the presence of adequate amounts of thymidine and vitamin B_{12} red blood cells are produced.

According to Glass, Boyd, Rubinstein, and Svigals, vitamin B_{12} is the extrinsic factor in pernicious anemia. The intrinsic factor, they believe, is a mucoprotein produced by the mucous cells of the gastric glands. They believe that the mucoprotein in some way aids in the absorption of vitamin B_{12}. It is found that vitamin B_{12} is much more effective when given to pernicious anemia patients by injection than by mouth. Large doses are necessary when given by mouth but when given together with mucoprotein, much smaller doses are effective.

Secondary Anemia. Many times anemia develops as a result of some other disturbance in the body; and, if the cause is removed, the anemia disappears. Such an anemia is called a secondary anemia. For example, if a person has a severe hemorrhage, he will be left in an anemic condition. Cancer and various types of infection may be accompanied by a secondary anemia. Intestinal parasites like tapeworms and hookworms produce anemia.

In the consideration of milk as a food it was pointed out that milk is deficient in that it is low in iron. Infants kept too long on a diet of milk alone become anemic. A common method of producing anemia in rats is to feed them for a long period of time on a milk diet. Anemia resulting from lack of iron in the diet is called **nutritional anemia.** Such anemias respond readily to treatment with iron salts.

In order to utilize iron in the synthesis of hemoglobin, it has been found that small amounts of copper must be present. Apparently copper acts as a catalyst in the synthesis of hemoglobin.

Blood Plasma. The discussion up to this point has dealt chiefly with the formed elements in the blood. We have now to consider the blood plasma. Of chief interest in this connection are the plasma proteins, the most important of which are **albumin,** various **globulins,** and **fibrinogen.** These constitute about 7 per cent of the blood plasma. Of these proteins 58 per cent is albumin, 38 per cent globulins, and 4 per cent fibrinogen.

Albumin and Globulin. Important functions of the proteins in the blood are to increase its viscosity and its osmotic pressure. The osmotic pressure of blood plasma is normally 7.26 atmospheres, of which 7.23 atmospheres are due to crystalloidal substances and 0.037 atmosphere to proteins. This slight osmotic pressure due to proteins at first sight appears to be insignificant, but in reality it is very important in maintaining the proper amount of water in the blood. Whereas the crystalloidal materials diffuse readily through the walls of the blood vessels, the proteins do not. Therefore the proteins produce a slight, though important, positive balance of osmotic pressure within the blood vessels. The fact that the blood proteins are hydrophilic colloids also contributes to their water-holding capacity.

Although all the blood proteins aid in the water-holding capacity of the blood, albumin is especially important in this capacity for two reasons: first, because it is present in the highest concentration; and, second, because it has the lowest molecular weight of any of the blood proteins and therefore has the greatest osmotic effect per unit of weight.

During World War II large quantities of blood plasma were used in military medicine for the treatment of burns and combating shock. In severe burns death may result from the loss of blood proteins. Replacing the proteins by the introvenous injection of blood plasma may save life. The purpose of blood plasma in shock is largely to restore or maintain the blood volume and thus keep up normal circulation. Since these effects are due mainly to the albumin present in the plasma, better results may be obtained by using solutions of blood albumin. At the present time blood plasma is divided into several protein fractions, each of which has its own special value.

Another use of plasma proteins is in a disease known as **nephrosis**, in which large amounts of albumin are excreted in the urine, with the result that the amount in the blood plasma is greatly reduced. In this condition water leaves the blood and enters the tissues, causing a swelling known as **edema.** Since in nephrosis there is no failure of the kidney to excrete nitrogenous wastes, it is possible to prevent edema in this disease by feeding a high-protein diet. The diet compensates for the loss of protein in the urine and tends to return the blood-plasma proteins to their normal level. When the blood-plasma proteins are less than 5 grams per 100 ml. edema results.

Several globulins have been isolated from blood plasma. The ones most generally recognized are euglobulin and α-, β-, and γ-globulins.

Euglobulin is a true globulin in that it has the characteristic solubilities of a globulin. It is soluble in dilute salt solution and is precipitated by one-third saturation of its solution with ammonium sulfate. Euglobulin appears either to be the isoagglutin which causes the agglutination of red blood cells during blood typing or to be associated with it. By separating the euglobulin from blood plasma it has been possible to obtain a solution which is thirty times more potent for use in blood typing than the original blood serum.

The **α-** and **β-globulins** are rich in lipids. It is thought that they are associated with the solution and transport of lipids in the blood. Of the proteins in blood plasma 14 per cent is α-globulin, and 13 per cent β-globulin.

The **γ-globulin** fraction of blood plasma is closely associated with defense against disease. When an individual contracts a disease, he usually becomes immune to further attacks of the same disease. This immunity appears to be carried in the γ-globulin fraction of the blood proteins. Certain diseases, such as measles, may be prevented or their severity lessened by injecting a child with blood serum from a person who has recovered from the disease. Here again the γ-globulin fraction of the serum is much more potent than the whole serum. γ-Globulin is being used to prevent infantile paralysis. Thirteen per cent of the plasma proteins are γ-globulin.

Blood Clotting. A very important property of blood is its ability to clot when removed from the blood vessels. If blood did not clot, an animal would bleed to death from a slight wound. Why blood does not normally clot in the blood vessels but does clot after it has been let is a subject that has been extensively studied. Many theories have been advanced to explain the many facts which are known concerning what happens when blood clots, but none seem to be entirely satisfactory. One theory suggested by Howell appears to account for the main facts. According to Howell clotting is due to the action of an enzyme, **thrombin,** which converts the soluble **fibrinogen** of the blood into insoluble **fibrin.** The reason blood does not clot in the veins is that thrombin does not exist in an active form in unlet blood, but is present as a pro-enzyme called **prothrombin.** Prothrombin is prevented from changing to thrombin by a constituent of the blood called **heparin,** which is combined with prothrombin in unlet blood. When blood is let, **thromboplastin,** a conjugated protein containing cephalin, is liberated from disintegrating blood platelets or injured tissue cells. Thromboplastin then unites with heparin, liberating prothrombin, which reacts with **calcium,** forming thrombin. Thrombin then acts on fibrinogen to form fibrin, which is the clot. What has been said may be represented diagrammatically thus:

$$\begin{tikzcd}
 & \text{Prothrombin-heparin complex} \arrow[d, "\text{Thromboplastin from platelets}"] \\
\text{Prothrombin} \arrow[d, "\text{Ca}"] & + \text{ Heparin-thromboplastin complex} \\
\text{Thrombin} & + \text{ Fibrinogen} = \text{Fibrin}
\end{tikzcd}$$

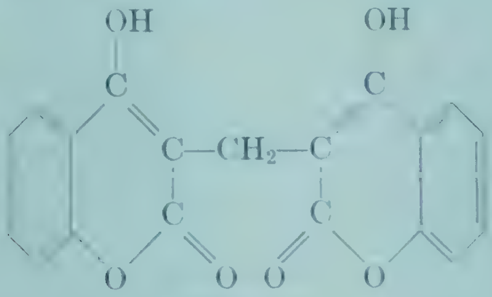
It will be noted that calcium is necessary for blood to clot. The usual method for preventing drawn blood from clotting is to add something which removes the calcium ion. An oxalate is the most common agent. This precipitates the calcium as calcium oxalate. In blood transfusions a citrate is used, since an oxalate is poisonous. Citrates do not precipitate the calcium; they merely depress its ionization. Since it is the calcium ion which is essential for blood clotting, citrates serve the same purpose as oxalates.

Sometimes blood clots in the blood vessels. Such intravascular clotting is called **thrombosis.** The danger from thrombosis is that the blood clot may become detached from the place where it is formed and be carried to some vital organ such as the heart or brain. If the clot becomes lodged in these organs death may occur quickly. The cause of thrombosis is not known. It has been suggested that injury to a blood vessel may cause the liberation of enough thromboplastin to cause clotting.

Several naturally occurring substances are known that prevent clotting. **Heparin,** which has already been mentioned, is a powerful anti-clotting agent used in the prevention and treatment of thrombosis. It is prepared from liver. Chemically, it is related to mucoitin sulfuric acid found in the mucin of the saliva. It gives glucuronic acid, glucosamine, and sulfuric acid on hydrolysis. Heparin is sometimes called an **antiprothrombin,** because it prevents the conversion of prothrombin into thrombin by thromboplastin.

Another substance that prevents blood from clotting is found in the salivary glands of the medicinal leech and is called **hirudin.** When a leech sucks blood hirudin is excreted by its salivary glands which prevents the blood from clotting in its digestive tract. Hirudin is protein in nature and appears to be a proteose. It inhibits the action of thrombin and is often referred to as an **antithrombin.**

A substance called **dicumarol** has been isolated from sweet clover which prevents blood from clotting.



Dicumarol

Cattle fed on spoiled sweet clover hay often die from internal hemorrhage. Dicumarol is used as a rat poison. It is mixed with the rat bait and fed to rats over a period of a week or two after which the rats die of internal hemorrhage. It is considered a safe rat poison because it must be taken continuously for several days to be effective. An animal accidentally eating the poisoned food once will not die.

Dicumarol is used in medicine to prevent thrombosis. It is said to function by interfering with the formation of prothrombin by the liver.

Vitamin K in the diet is essential in order that blood may clot in the normal time. It is necessary for the formation of prothrombin by the liver. Vitamin K is often given to a patient before surgery in order to prevent excessive hemorrhage.

Normally blood clots in about 5 minutes. In jaundice and certain liver diseases the time required for clotting may be much longer. In such cases Vitamin K is helpful.

In a rare disease known as **hemophilia** the clotting time of blood is increased greatly or it may not clot at all. This is an inherited disease which appears only in the male but is inherited from the female. Vitamin K is of no value in this disease. Hemophilia is thought to be caused by a lack of thromboplastin.

Before leaving the subject of blood clotting it is well to consider the difference between **blood plasma** and **blood serum,** two terms that are often confused. If blood is drawn, in the presence of an oxalate to prevent clotting, and is allowed to stand in a test tube the cells will settle out leaving a straw-colored liquid on top called plasma. If blood is drawn, without oxalate, and placed in a test tube it will clot. On standing the clot contracts leaving a straw-colored liquid on top called serum. Plasma is the liquid part of the blood and contains fibrinogen. If an oxalate is used to prevent clotting plasma should contain no calcium. Serum is the liquid part of the blood with no fibrinogen.

Tests for Blood. It often becomes necessary to test for blood in biological materials. The presence of blood in the urine, stomach contents, or feces is of great diagnostic importance. In medico-legal work it is often important to determine not only the presence of blood but also the kind.

Simply to test for the presence of blood is not difficult. Red blood cells can be readily identified under a microscope. Also a solution of blood can be easily detected by spectroscopic examination. Chemically the simplest procedure is to use the **guaiac** or **benzidine test.** In these tests the sample containing blood is treated with a solution of guaiac or benzidine in glacial acetic acid. If blood is present, a blue color develops upon the addition of hydrogen peroxide. These tests are sometimes objected to in medico-legal work on the grounds that other substances, such as milk, saliva, and plant juices, give the test. It will be recalled that the enzyme catalase gives these tests. (See p. 202.) It has been found that heating the sample for a few seconds before the test is made destroys the catalase but does not interfere with the test for blood.

The best chemical test for blood is the **hemin crystal test.** In this test the sample is placed on a microscope slide and heated with a physiological sodium chloride solution and glacial acetic acid. On cooling, characteristic brown crystals are formed if blood is present. This test gives no indication of the kind of blood.

To determine the kind of blood present in a blood stain, the **precipitin** and **complement fixation tests** are used. In the precipitin test the sample is dissolved in physiological salt solution and treated with a specific antiserum. To prepare human antiserum a rabbit is injected with repeated doses of human blood serum. In time the rabbit's blood serum develops the power of forming a precipitate with a very dilute solution of human blood but with no other blood. Other antisera may be prepared similarly by injecting rabbits with the blood serum of other animals.

To test for human blood a 1–1000 dilution of the sample is placed in a small test tube, and a few drops of human antiserum are added. If the sample contains human blood, a precipitate appears on standing in an incubator.

It should be pointed out that the precipitin test is a test for specific blood-serum protein and not necessarily for whole blood. In medicolegal work it is customary first to prove the presence of blood by the hemin crystal or some other test. The precipitin test then proves the origin of the blood.

Respiration. One of the main functions of the blood is to carry oxygen to and remove carbon dioxide from the tissues. The physiological process by which this is done is called respiration. In the lungs the blood is exposed to the oxygen of the inspired air. A very small amount of oxygen is physically dissolved in the blood, the main factor in the transport of oxygen being hemoglobin with which it forms an unstable chemical combination.

Normal blood will bind, as oxyhemoglobin, about 18.5 ml. of oxygen per 100 ml. The amount of oxygen bound by hemoglobin depends upon the amount of oxygen in the air to which the hemoglobin is exposed. The partial pressure of oxygen in the alveoli of the lungs is equal to about 108 mm. of Hg. In venous blood as it enters the lungs, the oxygen tension is about 50 mm. Thus oxygen passes by diffusion into the blood where it attains a tension of about 100 mm. in arterial blood. As arterial blood passes to the tissues where the oxygen tension is 50 mm. or less it gives up some of its oxygen to the tissues and becomes venous blood with an oxygen tension of 50 mm. The venous blood then returns to the lungs for more oxygen and the cycle is repeated. Blood leaving the tissues or, rather, returning to the lungs, contains about 12 ml. of oxygen per 100 ml. Thus 100 ml. of blood in circulating through the body supplies about 6.5 ml. of oxygen to the tissues.

As blood circulates through the body, it takes up carbon dioxide which is being produced as a result of oxidations going on in the tissues. Oxyhemoglobin is an unstable compound which tends to break down into oxygen and hemoglobin in the presence of carbonic acid. Thus, as carbon dioxide is taken up by the blood, oxyhemoglobin yields more oxygen to the tissues. That is to say, conditions in the tissues favor the decomposition of oxyhemoglobin.

The effect of carbon dioxide on the ability of hemoglobin to bind oxygen is shown in Fig. 30. The top curve shows the percentage saturation of hemoglobin with oxygen at various oxygen tensions ranging from 0 to 100 mm. where the carbon dioxide tension is 0 mm. The lower curve is for a carbon dioxide tension of 40 mm. At 100 mm. of oxygen tension hemoglobin in the absence of carbon dioxide is completely saturated with oxygen; at 40 mm. of carbon dioxide tension it is nearly saturated. At 40 mm. of oxygen tension the hemoglobin in the absence of carbon dioxide is 96 per cent saturated; at 40 mm. of carbon dioxide tension it is only 72 per cent saturated. Thus it is apparent that the presence of carbon dioxide depresses the ability of hemoglobin to bind oxygen, especially at the oxygen tension found in venous blood. Therefore in the tissues oxyhemoglobin releases some of its oxygen. The curves also show that the ability of hemoglobin to bind oxygen is dependent on the oxygen tension to which it is exposed.

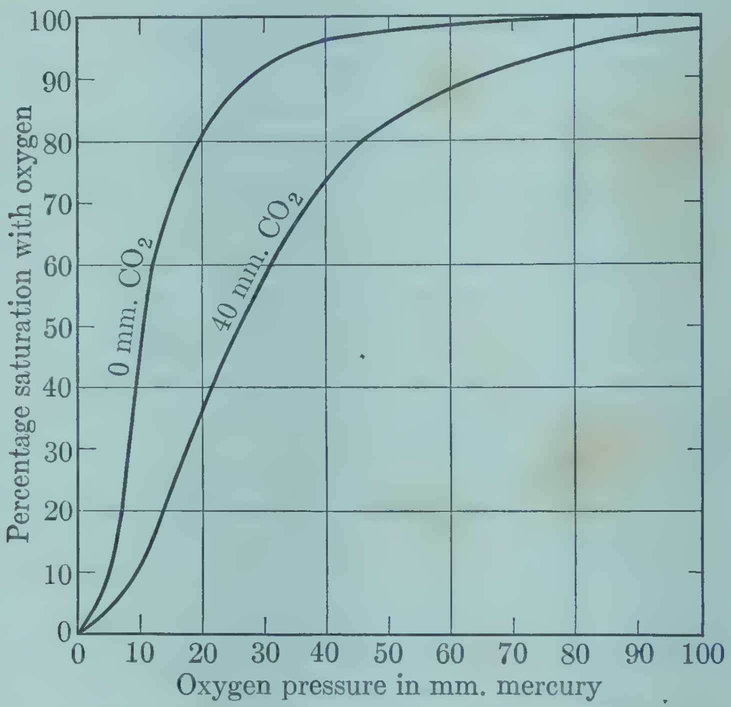
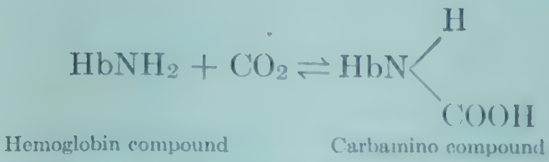


FIG. 30. Percentage saturation of human blood with oxygen when exposed to different oxygen and carbon dioxide pressures.

We may next inquire how the blood carries carbon dioxide from the tissues to the lungs and how the lungs eliminate it. Blood entering the lungs contains from 55 to 60 ml. of carbon dioxide per 100 ml.; that leaving the lungs contains about 50 ml. Thus in passing through the lungs 100 ml. of blood loses from 5 to 10 ml. of carbon dioxide.

There are three main ways in which the blood carries carbon dioxide. About 10 per cent is carried as dissolved carbon dioxide, about 20 per cent is held in combination as a hemoglobin compound, and about

70 per cent is in the form of $NaHCO_3$. The hemoglobin compound with carbon dioxide is a carbamino compound whose formation may be represented thus:



$$\underset{\text{Hemoglobin compound}}{HbNH_2} + CO_2 \rightleftharpoons \underset{\text{Carbamino compound}}{HbN\begin{matrix}H\\COOH\end{matrix}}$$

It will be noted that the above reaction is reversible. In the presence of reduced hemoglobin as found in venous blood the reaction goes from left to right, thus favoring the transport of carbon dioxide. In the presence of oxyhemoglobin as found in the lungs the reaction goes from right to left, thus favoring the release of carbon dioxide in the lungs.

The Chloride Shift. In the tissues carbon dioxide is constantly being produced as a result of the oxidation of food materials. The carbon dioxide dissolves in the tissue fluids and passes by diffusion into the blood plasma. From here it diffuses into the blood cells. As mentioned above, in the red blood cells some unites with hemoglobin to form a carbamino compound but most reacts with water to form carbonic acid. The formation of H_2CO_3 is hastened by an enzyme called **carbonic anhydrase,** which is present in red blood cells. The H_2CO_3 ionizes to form H^+ and HCO_3^- ions.

Red blood cells are rich in potassium, whereas blood plasma is rich in sodium. In the red blood cells CO_2 is present mainly as $KHCO_3$, in the plasma as $NaHCO_3$. The $KHCO_3$ in the red blood cells results from the reaction of H_2CO_3 with the KHb.

$$H_2CO_3 + KHb \rightarrow HHb + KHCO_3$$

The membrane of the red blood cells is permeable to H_2O, CO_2, HCO_3^-, Cl^-, and H^+; it is impermeable to K^+ and Na^+. As the concentration of HCO_3^- ions increases in the red blood cells, HCO_3^- ions diffuse out into the blood plasma. To compensate for the HCO_3^- ions lost Cl^- ions diffuse in. The above reactions take place in the capillaries of the tissues.

In the capillaries of the lungs the reactions described above are reversed. The hemoglobin-carbamino compound is broken down forming CO_2, and H_2CO_3 is decomposed by carbonic anhydrase also forming CO_2. As CO_2 is removed from the red blood cells in the lungs the HCO_3^- ion concentration in the cells becomes less than that in the plasma and HCO_3^- ions from the plasma diffuse into the cells. At the same time Cl^- ions diffuse out to maintain ionic equlibrium. The diffusion of Cl^- ions in and out of red blood cells to maintain ionic equilibrium is called the chloride shift. Figure 31 shows diagrammatically the chemical changes which take place in the blood of the lungs during respiration.

It is an interesting fact that during respiration two reactions are going on simultaneously in the tissues and in the lungs, each of which favors the other. In the tissues oxygen is removed from the red blood cells, because of the decomposition of oxyhemoglobin, and carbon dioxide, resulting from the oxidation of organic matter in the tissues, is removed from the tissues by the blood. The presence of carbon dioxide favors the breakdown of oxyhemoglobin as discussed above. Reduced hemoglobin is less acidic than oxyhemoglobin and favors the formation of the hemoglobin-carbamino compound thus favoring the taking on of carbon dioxide. In the lungs the reverse situation exists. As carbon dioxide is removed oxyhemoglobin is more easily formed. The formation of oxyhemoglobin favors the breakdown of the carbamino compound forming carbon dioxide.

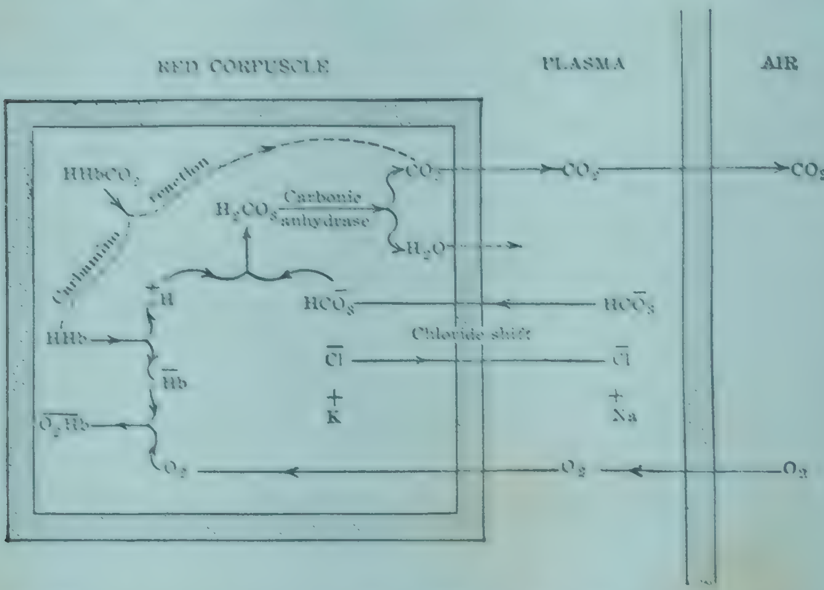

RED CORPUSCLE
PLASMA
AIR
$HHbCO_2$
Carbamino
reaction
H_2CO_3
Carbonic
anhydrase
CO_2
CO_2
CO_2
H_2O
$\overset{+}{H}$
$HC\overline{O}_3$
$HC\overline{O}_3$
Chloride shift
$\overline{Cl}$
$\overline{Cl}$
$\overset{+}{K}$
$\overset{+}{Na}$
HHb
$\overline{Hb}$
$\overline{O_2Hb}$
O_2
O_2
O_2

Fig. 31. The chemical changes taking place in the blood of the lungs during respiration. From *Physiological Reviews*. Courtesy of Dr. F. J. W. Roughton.

Acid-Base Balance. The blood is faintly alkaline, having a *p*H of about 7.35 under normal conditions. If the *p*H is altered by a few tenths, death results. If the *p*H is less than 7.35, a condition of **acidosis** is said to exist. If it is more than 7.35, the condition is known as **alkalosis.** The blood maintains its *p*H by means of certain constituents called **buffers.** Buffers are substances which, when in solution, will neutralize acids or bases so that the pH of the solution is not altered appreciably. The buffers in the blood are weak acids, together with their salts. An important buffer system in the blood is a mixture of sodium bicarbonate and carbonic acid, which may be represented by the ratio $[NaHCO_3]:[H_2CO_3]$. The $NaHCO_3$ will react with a strong acid to form the very weak acid H_2CO_3 and the salt of the strong acid.

$$NaHCO_3 + HCl \rightarrow H_2CO_3 + NaCl$$
$$H_2CO_3 \rightarrow H_2O + CO_2$$

If the foregoing reactions take place in the blood, it is evident that the strong acid, HCl, will be eliminated as carbon dioxide by the lungs and that, unless enough HCl is present to react with all the buffer of the blood, the reaction of the blood will not alter appreciably. The ability of the blood to neutralize acid is known as its **alkali reserve** and may be measured by treating a known volume of blood plasma with sulfuric acid and measuring the volume of carbon dioxide evolved. Normal plasma, saturated with carbon dioxide at the carbon dioxide tension existing in the lungs, gives from 55 to 80 ml. of carbon dioxide per 100 ml. of plasma. In acidosis this volume is reduced. A value of 40 indicates mild acidosis; of less than 30, severe acidosis. At about 15 the individual is in coma.

The $[NaHCO_3]:[H_2CO_3]$ buffer present in the blood also acts as a buffer toward a base. Thus a strong base like NaOH is neutralized to form the weaker base, $NaHCO_3$.

$$H_2CO_3 + NaOH \rightarrow NaHCO_3 + H_2O$$

Any excess of $NaHCO_3$ may be eliminated in the urine.

The phosphate buffer system may be represented by the ratio $[Na_2HPO_4]:[NaH_2PO_4]$. Na_2HPO_4 is a weak base, and NaH_2PO_4 is a weak acid. With a strong acid Na_2HPO_4 reacts to form the weaker acid, and with a strong base NaH_2PO_4 reacts to form the weaker base, thus:

$$Na_2HPO_4 + HCl \rightarrow NaH_2PO_4 + NaCl$$
$$NaH_2PO_4 + NaOH \rightarrow Na_2HPO_4 + H_2O$$

Any excess of acid or basic phosphates is eliminated from the blood through the kidneys in the urine. Thus the kidneys play an important part in maintaining the alkaline reaction of the blood. The reaction of the urine is normally acid, the pH being in the neighborhood of 6.0. However, the pH of the urine is constantly changing and at times may be distinctly alkaline. The pH of the urine depends upon whether acid or alkali must be removed from the blood to maintain the pH of the blood at 7.35.

Important buffers within the blood cells are hemoglobin and oxyhemoglobin. These buffers may be represented by the ratios $[\mathrm{KHb}]:[\mathrm{HHb}]$ and $[\mathrm{KHbO_2}]:[\mathrm{HHbO_2}]$. Other proteins in the blood act as a buffer system, represented by the ratio [Na protein]:[H protein].

Another means the body has of neutralizing acid is by the formation of ammonia. The urine contains a considerable quantity of ammonium salts. The ammonia for the production of these ammonium salts is formed in the kidney by the deaminization of amino acids. This is perhaps a mechanism for conserving base for the body. In acidosis the quantity of ammonium salts in the urine increases greatly. It is thus evident that ammonia may play an important role in regulating the pH of the blood.

Blood Analysis. In recent years methods have been developed for analyzing small quantities of blood for many of its constituents. Much work has been done to determine the concentration of the various constituents of blood in health and in disease. It has been found that the blood of normal individuals is quite constant in composition and that many diseases produce characteristic changes in this composition. Since, with the exception of carbon dioxide, most of the waste products of metabolism are eliminated by the kidneys, it is obvious that any failure of the kidneys to eliminate waste products of metabolism will result in an increase in the concentration of these products in the blood. Blood analysis therefore is of great importance in diagnosing kidney diseases and in determining the extent to which the kidneys have lost their function.

In a hospital laboratory the common determinations made in a chemical analysis of the blood are glucose, nonprotein nitrogen, urea nitrogen, uric acid, creatinine, and chlorides. A system of analysis by which all these constituents may be determined on one sample of blood has been developed by Folin and Wu. Blood is drawn from a vein in the arm into a tube containing a small quantity of potassium oxalate to prevent clotting. A sample of this oxalated blood is diluted with water, and the proteins are precipitated by means of tungstic acid. The proteins are removed by filtration, and the water-clear filtrate is analyzed for the various constituents. Table 10 shows the composition of normal human blood and blood from patients suffering from nephritis and diabetes.

Blood Chemistry in Nephritis. From Table 10 it is evident that blood composition varies from the normal in nephritis and diabetes. In ne-

TABLE 10

COMPOSITION OF HUMAN BLOOD IN HEALTH AND DISEASE

Constituent	Normal	Nephritis	Diabetes
	Milligrams per 100 ml.		
Nonprotein nitrogen	25–35	to 400	......
Urea nitrogen	10–15	to 300	to 30
Uric acid	2–3.5	to 27	to 10
Creatinine	1–2	to 28	to 4
Glucose	70–120	to 300	to 1200
Chlorides as NaCl	450–500	to 600	to 400
Cholesterol	150–190	to 900	to 800
Inorganic P (serum) (higher in children)	3–4	to 20	......
Calcium (serum)	9–11	5–7	......
Plasma CO_2 (volumes per cent)	55–80	to 45	10–50

phritis it is customary to determine first either nonprotein nitrogen or urea nitrogen. Nonprotein nitrogen is perhaps the better determination to make because of the technical difficulties involved in an accurate urea nitrogen estimation. It is obvious that, since nonprotein nitrogen is the total nitrogen of all the nitrogenous constituents of the blood filtrate, if this value is normal, the values for the other nitrogenous constituents will not be far from normal.

In early nephritis there may be a retention of uric acid. Since uric acid appears to be eliminated with more difficulty than any of the other blood constituents, if there is any impairment of kidney function, uric acid values may be high. In early nephritis it is therefore desirable to determine the uric acid in the blood even though nonprotein nitrogen values are apparently normal.

Of all the nitrogenous constituents comprising the nonprotein nitrogen of the blood, creatinine is the most easily eliminated. An increase of creatinine in the blood therefore indicates serious kidney impairment. If the nonprotein nitrogen value of blood is normal, it is a reasonable assumption that the creatinine value is normal. However, if the nonprotein nitrogen value is high, the creatinine value may or may not be high. It is therefore important to determine creatinine whenever blood with a high nonprotein nitrogen value is encountered. Normally blood contains from 1 to 2 mg. of creatinine per 100 ml. A value of 4 indicates serious kidney impairment. When the creatinine value reaches 5.5, there is usually little hope for the recovery of the patient.

Blood Chemistry in Diabetes. Another disease in which blood analysis is of great value is diabetes. Diabetes is usually first detected by finding sugar in the urine. Sugar in the urine is usually due to a high concentration of sugar in the blood. Normally, blood contains from

70 to 120 mg. of glucose per 100 ml. When the sugar in the blood reaches a value of 160 to 180, sugar appears in the urine. In severe diabetes the concentration of glucose in the blood may reach 1200 mg. per 100 ml. A blood-sugar estimation gives a much better indication of the severity of a diabetic condition than an estimation of sugar in the urine. A person with diabetes can often be brought by a proper diet to a condition where there is no sugar in the urine. When no sugar appears in the urine, the blood sugar may still be 160 to 180. Blood-sugar determinations are therefore important in controlling diabetic conditions in which blood-sugar values range from 120 to 180 mg. per 100 ml.

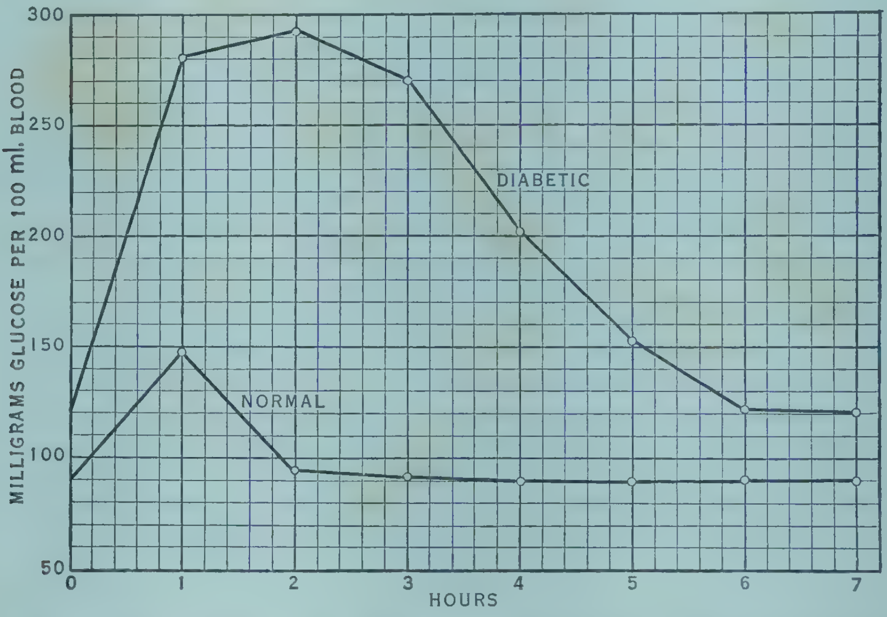


FIG. 32. Curves showing blood-sugar values before and at hourly intervals after a sugar meal for a normal and a diabetic individual.

Sugar-Tolerance Test. A very valuable application of blood chemistry is the sugar-tolerance test for diagnosing a diabetic condition. In this test the patient is given on an empty stomach 1.75 grams of glucose per kilogram of body weight. Blood samples are analyzed for glucose just before the sugar meal and at hourly intervals thereafter. The blood-sugar values are then plotted against time. In a normal individual the blood-sugar value rises to about 150 at the end of the first hour. In 2 hours the value is back to normal. In diabetes the blood-sugar values go higher (above 180) and remain high for several hours. The peak of the curve is reached in 2 hours, but it does not return to its original value until from 3 to 6 hours have passed. Figure 32 shows typical sugar-tolerance curves for normal and diabetic individuals.

Blood Chemistry in Other Diseases. Although blood chemistry finds its greatest use in the diagnosis and control of nephritis and diabetes, it should not be concluded that these are the only diseases in which it is of value. Reference has already been made to the importance of hemoglobin determinations in anemia, icteric-index determinations in jaundice, and carbon dioxide determinations in acidosis. In rickets, a disease characterized by poor calcification of bone, blood-serum phosphorus values are low. In gout there is a disturbance in purine metabolism which is associated with high uric acid values in the blood. One authority states that he never diagnoses a case as gout unless the uric acid of the blood exceeds 6 mg. per 100 ml. Blood-cholesterol values are high in diabetes, nephrosis, and obstruction of the bile duct. Values are low in pernicious anemia. Other examples of the applications of blood chemistry could be given.

REVIEW QUESTIONS

1. Name five functions of the blood.
2. How do blood, lymph, and blood plasma differ from each other?
3. Name the formed elements of blood.
4. What substances are present in blood plasma?
5. What is meant by laked blood?
6. What are normal values for a red- and for a white-cell count? How is each type of count made?
7. Describe the rulings on a counting chamber.
8. What is meant by a differential count? What are normal values?
9. In appendicitis how do the white-cell count and differential counts vary from normal?
10. How is the differential count altered from normal in lymphatic leukemia?
11. How are blood-platelet counts made? What is the function of blood platelets?
12. What is the chemical structure of hemoglobin?
13. What is the chemical structure of protoporphyrin?
14. How do oxyhemoglobin, methemoglobin, hematin, carboxyhemoglobin, hemin, and hemochromogen differ from hemoglobin in chemical structure?
15. What happens chemically in carbon monoxide poisoning? What are some of its symptoms and what treatment should be given?
16. Indicate the chemical steps in the formation of bilirubin from hemoglobin.
17. Indicate the chemical steps in the conversion of bilirubin into stercobilin.
18. What are the pigments in the urine? What is their origin?
19. Describe the Newcomer method for determining hemoglobin. According to Haldane how many grams of hemoglobin should there be in 100 ml. of normal blood?
20. Name and distinguish between two types of anemia.
21. What is meant by the color index?
22. What abnormal laboratory findings would you expect in pernicious anemia?
23. What is meant by the extrinsic and the intrinsic factors in pernicious anemia?

24. How may pernicious anemia be treated?

25. What vitamins are helpful in treating pernicious anemia and how are they thought to function?

26. How is nutritional anemia treated?

27. Name the important proteins found in blood plasma and give an important function of each.

28. What is the relation of blood proteins to edema?

29. Give Howell's theory of blood clotting.

30. Name three substances that inhibit blood clotting and indicate how each acts.

31. What is the function of vitamin K?

32. What is hemophilia?

33. What is thrombosis? What may cause it and how may it be prevented?

34. What is the difference between blood plasma and blood serum?

35. Name and describe several tests for blood. How can human blood be tested for?

36. Define respiration.

37. In what form is oxygen carried in the blood from the lungs to the tissues?

38. Name three forms in which carbon dioxide is carried in the blood. What percentage is carried in each form?

39. What effect does carbon dioxide have on the ability of hemoglobin to combine with oxygen?

40. What effect does oxygen have on the ability of hemoglobin to unite with carbon dioxide to form a carbamino compound?

41. What is meant by the chloride shift? Explain.

42. Name five buffer systems present in the blood. How does the blood maintain a constant pH value?

43. What are acidosis and alkalosis?

44. How are ammonium salts in the urine related to the acid-base balance of the body?

45. Discuss the value of blood chemistry in nephritis and diabetes.

46. Name several constituents of the blood commonly determined in a blood analysis and give normal values for each.

47. Describe a sugar-tolerance test. What results would you expect to find in a normal and in a diabetic individual?

48. Name some diseases other than diabetes and nephritis in the diagnosis of which blood chemistry is of value, and tell how the blood composition may vary from the normal in each.

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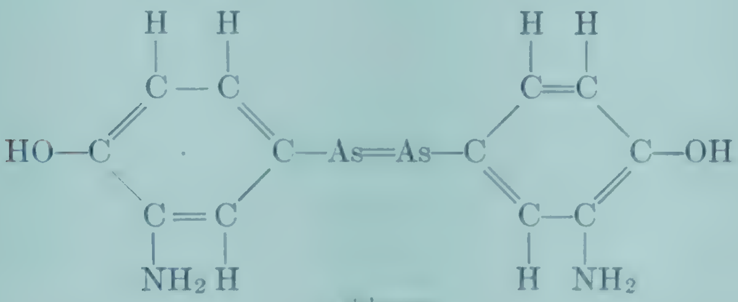
18

Chemotherapy and Antibiotics

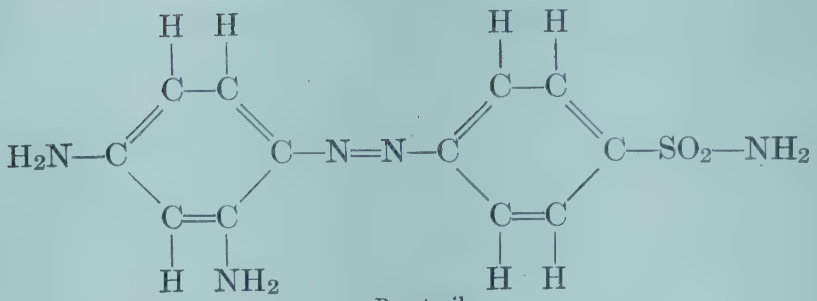
Ever since the discovery that many diseases are caused by the entrance of microorganisms into the body, it has been the hope of medical men that some drug or chemical might be found which, when introduced into the body, would destroy the microorganism but be harmless to the patient. It was soon realized that this was a difficult problem. Although many chemicals were known which would kill microorganisms, they all proved to be toxic to the patient also. However, some success was obtained in chemotherapy when it was shown that malaria could be controlled by the use of **quinine.** The real beginning of chemotherapy dates back to 1910, when Ehrlich showed that **salvarsan,** an arsenic-containing aromatic compound, will cure syphilis. Salvarsan is commonly called 606 to indicate the number of compounds Ehrlich tried before he found the right one.



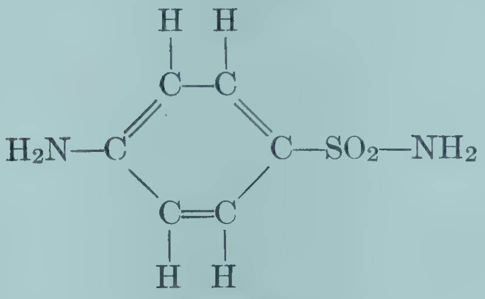
Salvarsan

Sulfonamides. One of the most important advances in chemotherapy was the development of the so-called **sulfa drugs.** These are all derivatives of **sulfanilamide,** which was first prepared by Gelmo in 1908. It was not until 1935 that its value as a drug became known.

In 1932 Domagk showed that a dye, prontosil, protects mice against hemolytic streptococcus. In 1935 Trefouel, Nitti, and Bovet in France showed that prontosil is converted into sulfanilamide in the body and that the sulfanilamide is the therapeutically active agent. In 1936 their work was confirmed in this country by Long, Bliss, and Marshall.



Prontosil



Sulfanilamide

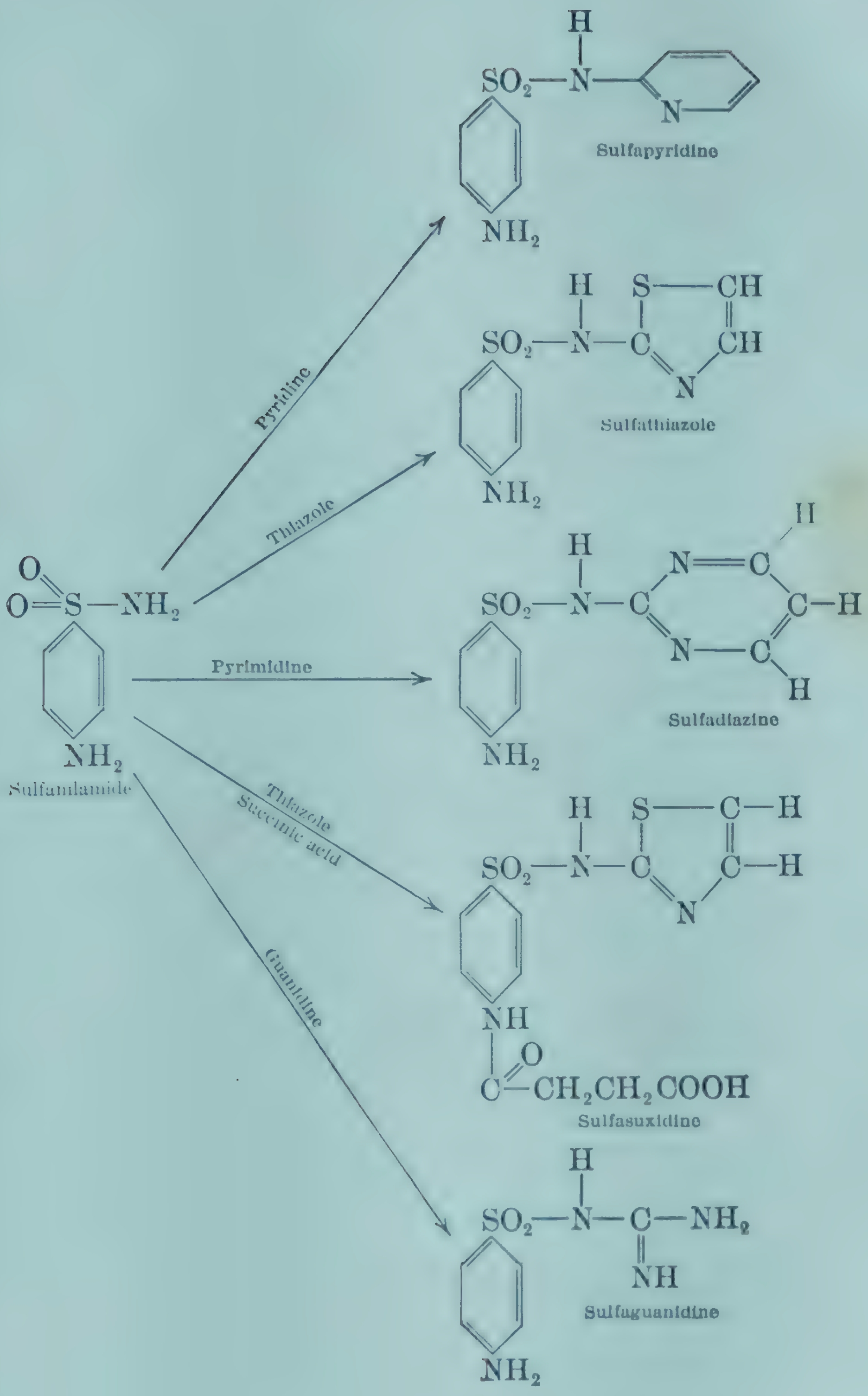
Sulfanilamide has proved to be effective against infections following childbirth, blood-stream infections, erysipelas, scarlet fever, mastoiditis, septic sore throat, gonorrhea, and streptococcal meningitis. It has proved of no value against typhoid fever, tuberculosis, rheumatic fever, influenza, colds, and staphylococcic infections.

Sulfa drugs should be used only under the direction of a physician, because serious reactions often follow their use. Some of the common reactions are loss of appetite, skin rashes, dizziness, psychoses, cyanosis, and anemia.

In an effort to produce sulfa drugs which are more effective and more easily tolerated than sulfanilamide scientists have prepared many derivatives of sulfanilamide and have studied their therapeutic activity. Among those which have been especially valuable are **sulfapyridine, sulfathiazole, sulfadiazine, sulfasuxidine**, and **sulfaguanidine.** Their chemical structures and their relationship to sulfanilamide are indicated in the diagram on p. 341.

Sulfapyridine is especially good for certain types of pneumonia. Sulfathiazole is effective against staphylococcic infections. Sulfadiazine is well tolerated and is very effective in pneumonia and staphylococcic infections. Sulfasuxidine and sulfaguanidine are poorly absorbed from the intestine and for this reason have been especially useful in intestinal infections.

The current belief concerning the mechanism by which sulfa drugs overcome bacterial infections, is that the drugs do not actually kill the organisms but rather slow down their activity, thus giving the body


Sulfanilamide
Pyridine
Thiazole
Pyrimidine
Thiazole
Succinic acid
Guanidine
Sulfapyridine
Sulfathiazole
Sulfadiazine
Sulfasuxidine
Sulfaguanidine

cells a chance to dispose of them. One theory states that bacterial cells require **p-aminobenzoic acid** for their normal metabolism and that the sulfa drugs, because their structures are similar to that of *p*-aminobenzoic acid, interfere with the normal function of this compound. The theory suggests that, before being utilized, *p*-aminobenzoic acid must become associated with an enzyme. If sulfanilamide is present it combines with the enzyme, thus leaving the *p*-aminobenzoic acid in a form that cannot be used by the bacterial cell. This is an example of a process known as **competitive inhibition,** in which two structurally similar molecules are both capable of combination with a specific enzyme. In the above instance, the enzyme can function properly only when associated with the *p*-aminobenzoic acid; if an unnatural molecule such as sulfanilamide, which is structurally similar to *p*-aminobenzoic acid, combines with the enzyme instead, the enzymatic reaction cannot proceed and is effectively inhibited. Another application of this principle in chemotherapy is the use of **isoniacin hydrazide** in the treatment of tuberculosis. This compound is an analogue of nicotinamide, a vitamin of importance in enzymatic oxidation and reduction reactions.

Antibiotics. In contrast to the sulfa drugs, which were developed synthetically, there is a large group of naturally occurring chemotherapeutic agents known as the antibiotics. As strictly defined, these are substances, produced by microorganisms, that are effective in inhibiting the growth of other microorganisms. Although antimicrobial substances have been isolated from higher plants, these are not regarded as antibiotics in the strictest sense of the word. One such example is the compound, **tomatin,** produced by the tomato plant.

Gramicidin and Tyrocidin. In 1939, Dubos obtained from cultures of *Bacillus brevis* an extract which he called **tyrothricin.** This preparation proved highly inhibitory to the growth of most bacteria against which it was tested and thus showed promise as a chemotherapeutic agent. Dubos was able to isolate two crystalline materials from the tyrothricin preparation. The first of these, **gramicidin,** is a water-insoluble substance which has been characterized as a cyclic polypeptide. It inhibits the growth of many Gram-positive bacteria but has found only limited therapeutic use because of its high toxicity when injected into animals. It may be administered orally without harming the animal and is used in this way in the treatment of many throat infections.

The second component isolated from tyrothricin was named **tyrocidin.** It is also a polypeptide and is highly inhibitory to almost all Gram-positive and Gram-negative bacteria against which it has been tested. Tyrocidin is inactivated by blood and body fluids in general and thus is ineffective *in vivo*.

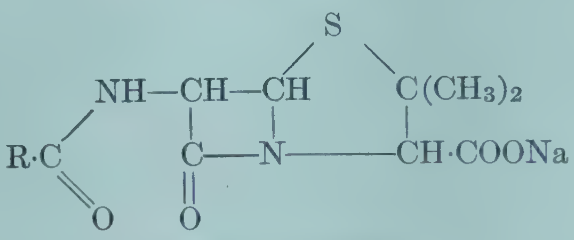
Even though these two antibiotic substances have had only limited practical application as therapeutic agents, the careful work involved in their isolation and characterization may perhaps be credited with stimulating an interest in the then little-known field of antibiotics.

Penicillin. Another important advance in chemotherapy was the discovery and large-scale production of a substance called penicillin, which is a product of the metabolism of a green mold called *Penicillium notatum*. Penicillin was first discovered in 1929 by Fleming in England. In working with plate cultures of staphylococci he noted that, when a culture was accidentally contaminated with green mold, growth of the staphylococci was inhibited. Further studies showed that filtrates from cultures of green mold also inhibit the growth of gonococci, pneumococci, and streptococci. The growth of *Escherichia coli* and of *Hemophilus influenzae* was not affected. Fleming called his green-mold filtrates penicillin.

Little attention was paid to Fleming's work until after the studies on tyrothricin were published. At the beginning of World War II, Abraham, Chain, Florey, and their associates, known as the Oxford group, started to reinvestigate penicillin with the idea of using it on war casualties. Because of the difficulties of working in England under war conditions and because of the urgency of the work, Florey came to the United States and interested the government and commercial laboratories in helping with the problem. As a result of this concerted effort, penicillin was soon being produced in quantity and used successfully in the treatment of many infectious diseases.

Penicillin is produced by growing the mold on a liquid culture medium. The liquid medium containing the penicillin is finally separated from the mold and concentrated by distillation under reduced pressure. The residue is extracted with organic solvents, and the penicillin finally precipitated as the barium salt. For clinical use the barium salt is converted into a solution of the sodium or calcium salt.

Since the discovery of penicillin much work has been done by British and American chemists to determine its chemical nature. This work indicates that there are several penicillins, which have the empirical formula $C_9H_{11}O_4SN_2 \cdot R$. The various penicillins differ with regard to the nature of R. In F-penicillin (known in Britain as penicillin I), R is Δ^2-pentenyl ($—CH_2 \cdot CH{=}CH \cdot CH_2 \cdot CH_3$); in dihydro-F-penicillin, R is *n*-amyl; in G-penicillin (known in Britain as penicillin II), R is benzyl; in X-penicillin (known in Britain as penicillin III), R is *p*-hydroxybenzyl; and in K-penicillin, R is *n*-heptyl. The following has been suggested as the graphic formula for the sodium salt of penicillin:

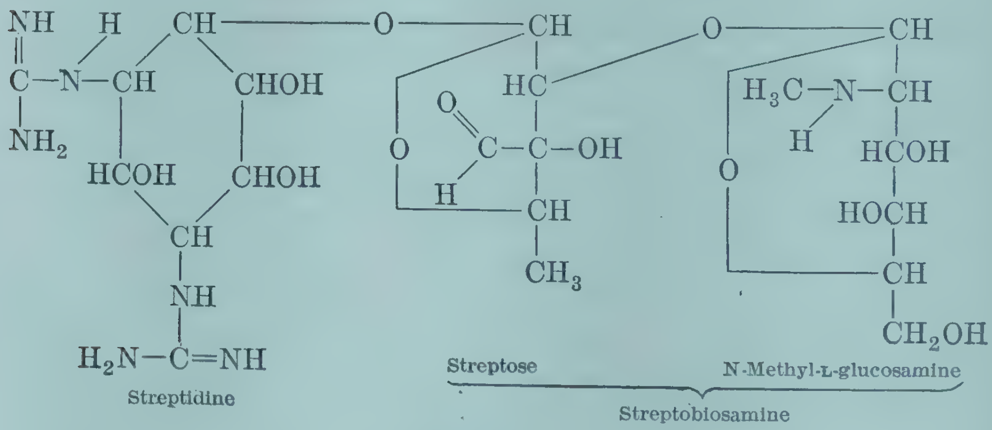

S
NH—CH—CH
C(CH3)2
R·C
C—N—CH·COONa
O
O

Sodium salt of penicillin

Penicillin is administered by intravenous, intramuscular, or subcutaneous injection. For application to surface wounds the calcium salt is used, because it is more stable than the sodium salt. Since penicillin is quickly eliminated from the body by the kidneys, frequent doses must be given.

In comparison with the sulfa drugs, penicillin is much less toxic. It is generally well tolerated by humans even when administered in large doses. Penicillin acts on many Gram-positive microorganisms, including the staphylococci, against which the sulfa drugs are relatively ineffective.

Streptomycin. In 1944, Waksman, Bugie, and Schatz isolated an antibiotic from cultures of a soil organism called *Streptomyces griseus*, which belongs to the group of microorganisms known as the actinomycetes. This compound, which they named streptomycin, has found wide use because of its effectiveness against a large number of pathogenic bacteria, including many Gram-negative forms. In 1952 Waksman received a Nobel prize for his work on streptomycin.


NH
H
CH
O
CH
O
CH
C—N—CH
CHOH
HC
H3C—N—CH
NH2
O
C—C—OH
H
HCOH
HCOH
CHOH
O
O
H
CH
HOCH
CH
CH
CH3
NH
CH
H2N—C=NH
CH2OH
Streptidine
Streptose
N-Methyl-L-glucosamine
Streptobiosamine

Streptomycin

Largely through the efforts of a group of chemists at Merck and Company, the structure of the compound has been elucidated. The molecule is composed of three segments joined by oxygen bridges.

These linkages may be broken by acid hydrolysis so that three distinct compounds result. As indicated in the formula, these three segments, in order, are **streptidine, streptose,** and **N-methyl-L-glucosamine.** The last two compounds make up a disaccharide called **streptobiosamine.** The student will recall that glucosamine is frequently encountered in nature and is especially prominent as a constituent of animal connective tissue. It should be noted that the glucosamine is of the L variety in streptomycin.

Some cultures of *Streptomyces griseus* produce a less potent derivative of streptomycin which is known as **mannosidostreptomycin.** In this molecule, D-mannose is attached to the N-methyl-L-glucosamine portion of the molecule through a typical sugar linkage. Cultures of *Streptomyces griseus* which normally produce streptomycin are capable of hydrolyzing this derivative to streptomycin and D-mannose, apparently through the action of an enzyme which has been named mannosidostreptomycinase.

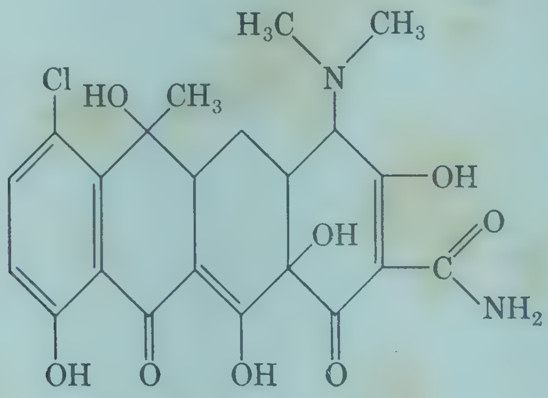
Catalytic hydrogenation of streptomycin causes the reduction of the free aldehyde group of the streptose portion of the molecule, resulting in the formation of **dihydrostreptomycin.** This derivative is similar to streptomycin in its antibacterial activity but appears to be more stable chemically and also less toxic to the animal than streptomycin. It is possible that dihydrostreptomycin is oxidized back to streptomycin by the organisms against which it is effectively used. This would explain the similarity in antibiotic potency of the two compounds.

There was great hope in the early days of the development of streptomycin that it would unequivocally cure tuberculosis by eliminating the tubercle bacillus. It is now thought, however, that this antibiotic merely suppresses the growth of this organism, thereby retarding the disease process. Unfortunately, some strains of this pathogen have developed a resistance to streptomycin. In cases in which such organisms are involved in the production of the disease, streptomycin has limited therapeutic value.

Streptomycin is required in fairly high doses to inhibit the growth of the nonhemolytic streptococci and the hemolytic staphylococci. Although for the most part, streptomycin in these doses produces no adverse effect in humans, instances have been reported in which the development of a measles-like rash was attributed to this antibiotic. Higher doses of streptomycin, administered over extended periods, may damage the auditory nerve and have been known to lead to permanent deafness in humans.

Streptomycin and penicillin show differences in the organisms against which they may effectively be used. However, when employed against organisms that they both inhibit, the two drugs often show an additive effect.

Aureomycin. In 1948, Duggar discovered an antibiotic, produced by a new species of *Streptomyces*. He proposed the name, *Streptomyces aureofaciens* for this organism, since at certain times in its development, a golden-yellow pigment is produced. The antibiotic also has a faint-yellow color, and thus he named it aureomycin. According to Woodward and other workers in the Chas. Pfizer and Company laboratories the molecular structure of aureomycin is as shown.



Aureomycin

The discovery of this antibiotic was immediately heralded as a great advance in the field of chemotherapy, for it was the first such substance to show promise in the treatment of virus and rickettsial diseases. It has now been used successfully in the rickettsial infections of Q fever, Rocky Mountain spotted fever, Brill's disease, rickettsial pox, and murine typhus. The effectiveness of aureomycin against virus diseases is less conclusive. It has been reported to be helpful in cases of chicken pox, small pox, measles, and hepatitis; it has been of questionable value in the treatment of some cases of virus influenza and atypical pneumonia. It is apparently of no benefit in the treatment of mumps.

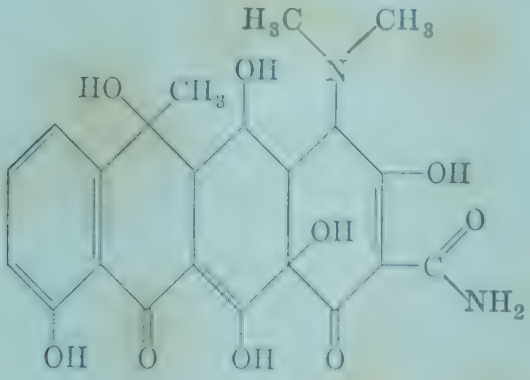
Certain spirochetal and protozoal infections may be successfully treated with aureomycin. It is also effective against many pathogenic bacteria and is widely used when penicillin and streptomycin have lost their potency owing to the development of resistant strains of bacteria.

Aureomycin remains active after oral administration and is slowly eliminated so that therapeutic concentrations may be maintained for 6 to 8 hours after the administration of a single dose of 300 to 700 mg. in adults.

Terramycin. The antibiotic terramycin was first isolated in the laboratories of Chas. Pfizer and Company, Inc. Its discovery was the result of a tremendous research program in which thousands of soil samples from all over the world were analyzed for organisms that produced antibiotic substances. Terramycin is the product of the metabolism of *Streptomyces rimosus*, an organism first discovered as a result of this intensive project of soil analysis.

Terramycin is an amphoteric substance and forms crystalline acidic and basic salts. Its molecular formula is similar to that of aureomycin. These two drugs are also quite similar in their activity as antibiotics.

According to Woodward and other workers in the Chas. Pfizer and Company laboratories the structural formula for terramycin is

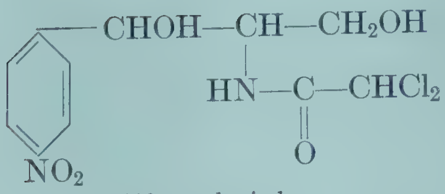


Terramycin

Terramycin is active against many Gram-positive and Gram-negative bacteria, the tubercle bacillus, and numerous rickettsias, and has found wide use in the treatment of brucellosis, gonorrhea, trench mouth, and bacillary dysentary. Less conclusive results have been obtained in the treatment of influenza virus, pneumococcus meningitis, and whooping cough. It has proved helpful in cases of syphilis, but more time must be allowed to assess its true value against this disease. Terramycin is effective after oral administration and seems to be relatively nontoxic to humans.

Chloramphenicol. This antibiotic, known commercially as **chloromycetin,** was discovered in 1947 in the soil of a field in Caracas, Venezuela, by Ehrlich, Burkholder, and co-workers. It is produced by a soil microorganism which has been named *Streptomyces venezuelae*. Chloramphenicol has the relatively simple chemical structure shown and has been prepared synthetically. It is one of the few known natural substances to contain an aromatic nitro group.

Chloramphenicol is notably effective against Gram-negative bacteria, principally those of the colon-typhoid group. It is perhaps the most helpful of the known antibiotics in the treatment of whooping cough.



Chloramphenicol

After rather extensive use of this drug, it was noted that in a very few cases its administration seemed to be associated with the production of a serious anemic condition. Until further studies clarify this relationship, the use of this antibiotic has been greatly restricted.

Polypeptide Antibiotics. In addition to gramicidin and tyrocidin, several other antibiotics which have been chemically identified as polypeptides have been isolated. In general, these drugs are toxic systemically, so their use is often limited to the treatment of skin infections.

Bacitracin, isolated from the Tracey strain of *Bacillus subtilis,* is a neutral polypeptide which is heat stable and water soluble. It is most effective when used against Gram-positive organisms. Bacteria sensitive to bacitracin are generally the same as those affected by penicillin, except that bacitracin more readily eliminates some staphylococci and certain nonhemolytic streptococci. It is relatively nontoxic but may produce damage to the renal tubules.

Bacitracin combines chemically with aureomycin to form **aureotracin.** This complex has all the antibacterial properties of each of the drugs alone but is less toxic than bacitracin.

Other polypeptide antibiotics are **polymyxin,** produced by *Bacillus polymyxa,* and **subtilin,** a product of *Bacillus subtilis.* The use of subtilin in food preservation has been suggested.

Mention has been made of only a few of the great number of antibiotics which have been isolated and characterized. An effort has been made to familiarize the student with those drugs that are commonly encountered in medical practice at the present time.

Mode of Action of Antibiotics. There is still a great deal of conjecture as to how the antibiotics interfere with the growth of certain microorganisms. Early studies on sensitive organisms indicate that penicillin blocks glutamic acid assimilation, that streptomycin interferes with the formation of citrate in the Kreb's carbohydrate oxidation cycle, and that chloramphenicol disrupts the normal metabolism of the aromatic amino acids. Whether or not the inhibition of these particular reactions will prove to be the principal effects of these drugs remains to be seen, but it seems quite likely that most of the growth inhibition observed is the result of interference of the antibiotic with some vital enzymatic process carried on by the susceptible organism.

Since comparative biochemistry teaches that these vital processes are often quite similar in microorganisms and in the higher animals, one wonders why the same reactions blocked in the metabolism of the microorganism are apparently not disturbed in the tissues of humans who undergo antibiotic therapy. The work of Umbreit and co-workers has suggested that the reason the antibiotics do not interfere with enzymatic reactions in animal tissues is that certain barriers exist in animal cells which prevent these drugs from reaching the vital enzyme systems. If animal cells are broken up, or homogenized, the antibiotic that Umbreit studied is able to block the same enzyme system with which it interferes in the intact bacterial cell.

The appearance of new strains of once-susceptible bacteria which have developed resistance to the action of the antibiotics would seem to be due to mutations in which other metabolic pathways have been developed that side-step the particular reaction blocked by the antibiotic. Resistant strains of organisms generally susceptible to antibiotic action have been reported for most of the antibiotics now in clinical use.

Combinations of Antibiotics. It has been shown that certain antibiotics when used together are more effective than when they are used singly. Chloromycetin and aureomycin, streptomycin and terramycin, streptomycin and penicillin, and bacitracin and penicillin are such combinations. On the other hand, penicillin and chloramphenicol antagonize each other with the result that the combination is much less effective than either of the agents used singly.

Antibiotics in Feeds. In the commercial production of aureomycin, *Streptomyces aureofaciens* is grown in a culture medium designed to allow the organism to produce maximum amounts of the antibiotic which is then extracted from the culture fluid. The remainder of the medium contains various other metabolic products of the organism, including vitamin B_{12}. It seemed quite likely that the presence of this vitamin in the culture medium might make it a valuable animal-feed supplement. Accordingly, the extracted residue was fed to experimental animals and, as predicted, did lead to better growth than that observed in control animals receiving no such supplement. However, it was soon found that the waste culture fluid owed its ability to stimulate animal growth not only to vitamin B_{12} but also to small amounts of aureomycin which had not been extracted from the medium. As a result of this discovery, many commercial animal feeds now contain aureomycin or other antibiotics that produce similar effects when used as dietary supplements.

It is not altogether clear just why aureomycin should stimulate animal growth, but it is generally believed that the antibiotic rids the animal of many bacteria which normally retard growth.

REVIEW QUESTIONS

1. What is meant by the term chemotherapy? Discuss the historical development of chemotherapy.
2. What is salvarsan, and against what disease is it used?
3. Name five sulfa drugs which are derivatives of sulfanilamide and indicate their chemical structure.
4. Name several diseases which respond to sulfa drug therapy and several which do not.
5. What sulfa drugs are especially good for combating intestinal infections? Why?
6. Give a theory to explain how sulfa drugs act.
7. What is the chemical nature of gramicidin and tyrocidin?
8. Discuss the historical development of penicillin.
9. How is penicillin made? What advantage does it have over sulfa drugs?
10. What is the chemical nature of penicillin?
11. What is the chemical structure of streptomycin? Of what three simpler molecules is it composed?
12. What are mannosidostreptomycin and dihydrostreptomycin? What is the advantage of the latter over streptomycin?
13. Discuss the use of streptomycin in tuberculosis.
14. What bad effects may result from large doses of streptomycin?
15. Against what types of diseases is aureomycin effective and penicillin and streptomycin ineffective?
16. Compare terramycin and aureomycin with regard to chemical composition and antibiotic activities.
17. What is the commercial name for chloramphenicol? What is its chemical structure?
18. Name several antibiotics that are polypeptides.
19. Discuss the mode of action of antibiotics. Why are they relatively harmless to animals?
20. Are combinations of antibiotics more effective than antibiotics used singly?
21. Discuss the use of antibiotics in feeds.

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19

Urine

Excretions. The main organs of the body concerned with the removal of the waste products of metabolism are the **kidneys** and the **lungs.** The main functions of the kidneys are to eliminate water, salts, and the waste products of metabolism and to regulate the *p*H of the blood and tissues. The kidneys eliminate the end products of metabolism which are solids or liquids; the lungs eliminate carbon dioxide and small amounts of other volatile substances. Other organs which have excretory functions are the **liver, intestines,** and **skin.** The liver excretes the bile pigments, bile salts, and cholesterol; the intestines, certain mineral salts; and the skin, through the perspiration, many of the compounds ordinarily found in the urine. Most of the nitrogenous waste products are eliminated through the kidneys. An adult on an average diet excretes in the urine about 15 grams of nitrogen per day. The nitrogen excreted by other routes amounts to about 1 gram per day. It is thus apparent that the urine is a very important excretion to be considered in the study of nitrogen metabolism.

Kidney Structure and Physiology. In order to understand the action of the kidney a brief description of the structure of this organ is necessary. In the kidney the waste products of metabolism are removed from the blood by what may be considered a process of filtration. A human kidney has been estimated to contain about 4,500,000 filtration units. Each filtration unit is known as a **Malpighian corpuscle.** The blood enters the Malpighian corpuscle through an afferent vessel which breaks up into a capillary nodule called the **glomerulus.** The capillaries of the glomerulus collect into one vessel, called the efferent vessel, which leads out of the Malpighian corpuscle. The glomerulus is enclosed within a capsule known as **Bowman's capsule.** Each Malpighian corpuscle is thus made up of two parts, a glomerulus and a capsule.

To each capsule is attached a long tubule. For a short distance from the capsule the tubule doubles and twists and then straightens out. This first region of the tubule is known as the **proximal convoluted tubule.** The straightened tubule descends for some distance and then doubles on itself, forming **Henle's loop.** The tubule then goes through

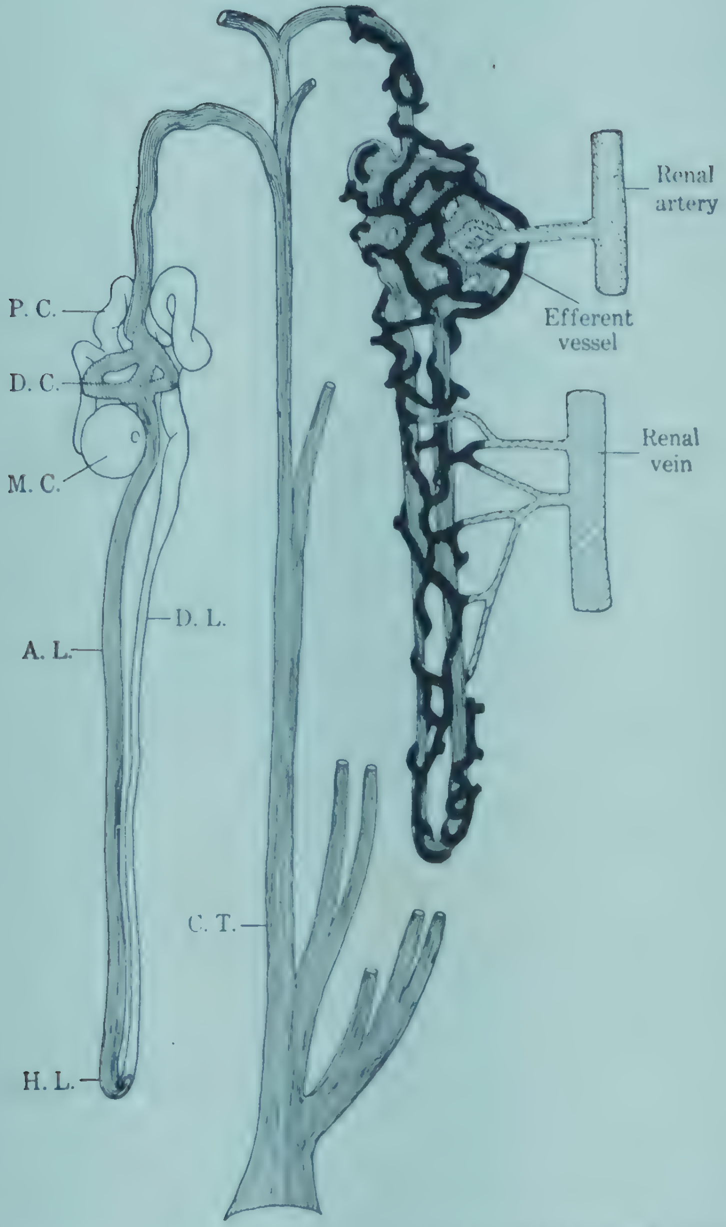

Renal
artery
Efferent
vessel
Renal
vein
P. C.
D. C.
c
M. C.
D. L.
A. L.
C. T.
H. L.

FIG. 33. Diagram of the tubular structure of the kidney. To the unit on the right the blood circulation is indicated. *M. C.*, Malpighian corpuscle; *P. C.*, proximal convoluted tubule; *D. L.*, descending limb of Henle's loop; *H. L.*, Henle's loop; *A. L.*, ascending limb of Henle's loop; *D. C.*, distal convoluted tubule; *C. T.*, collecting tubule. From *The Secretion of the Urine* by Cushny. Courtesy of Longmans, Green & Co.

another series of convolutions. This section of the tubule is known as the **distal convoluted tubule.** The tubule finally straightens out and ends in a **collecting tubule.** The blood supply to the tubules is a network of capillaries originating from the efferent vessel of the glomerulus. The accompanying diagrams (Figs. 33 and 34) illustrate the structure of the Malpighian corpuscle and also the relation of the Malpighian corpuscle to the tubule, together with its blood supply.

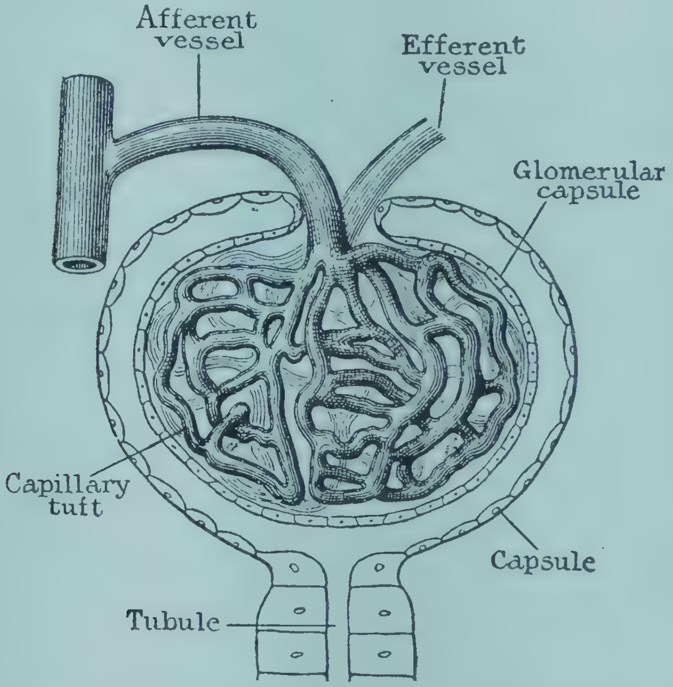


FIG. 34. A Malpighian corpuscle. From *The Secretion of the Urine* by Cushny. Courtesy of Longmans, Green & Co.

Many theories have been advanced concerning the formation of urine. Perhaps the most generally accepted is that, as the blood passes through the glomerulus, the noncolloidal substances in solution in the blood filter through the capillary walls forming what is known as the glomerular filtrate. As this filtrate passes down the tubules, it is concentrated by the reabsorption of water. Those substances in the filtrate that are of value to the body are reabsorbed along with the water. Such substances are known as **threshold substances;** among them are glucose, amino acids, and salts. Substances like urea, uric acid, and creatinine, which are of no further use to the body, are not absorbed by the tubules to any appreciable extent under normal conditions. Certain substances produced by the kidney, such as ammonium salts, and certain foreign substances, such as particular dyes, are excreted by the tubule cells directly into the tubules.

The degree to which the glomerular filtrate is concentrated in passing through the tubules can be determined by comparing the concentration of the various urinary constituents in the urine and in the blood. Urea is 60 times and creatinine 100 times more concentrated in the urine than in the blood. This fact indicates that the glomerular filtrate is reduced to 1 per cent of its volume in passing through the tubules.

The main factor determining the rate of filtration in the glomerulus is the blood pressure. If the osmotic pressure of the blood colloids is subtracted from the blood pressure, the remainder is the filtration pressure in the glomerulus. If the blood pressure falls below the osmotic pressure of the blood colloids, there will be no urine formation. The osmotic pressure of the blood colloids is equal to 30 to 40 mm. of mercury. In an experiment in which Starling stopped the flow of urine by obstructing a ureter he found the urinary pressure to be equal to 92 mm. of mercury when no more urine was being excreted. The blood pressure of the animal at this time was equal to 133 mm. of mercury. The difference in pressure, equal to 41 mm. of mercury, was the osmotic pressure of the blood colloids.

Volume. The volume of urine excreted in 24 hours varies considerably, the most important factor influencing it being the volume of water consumed. The average for a normal individual is about 1200 ml. In warm weather the volume is below normal because of loss of moisture through perspiration. Many drugs stimulate the kidneys, causing an increase in volume. Such drugs are said to have a **diuretic effect.** Perhaps the best example is caffeine, found in coffee and tea. Since urea and other end products of protein metabolism have a diuretic effect, the amount of protein in the diet influences the volume of urine.

In disease the amount of urine may vary widely from the normal. In acute nephritis the quantity may be greatly reduced, and in extreme cases there may be no urine at all. When the quantity of urine is small, the condition is known as **oliguria;** when there is no urine, the condition is known as **anuria.** Oliguria occurs not only in acute nephritis but also in heart disease, fever, diarrhea, and vomiting. Anuria occurs after poisoning by mercuric chloride. When the volume of urine is much above normal, the condition is known as **polyuria.** Polyuria occurs in **pancreatic diabetes** and to an extreme degree in **diabetes insipidus,** where the volume may exceed 20 liters per day. It also occurs in certain diseases of the nervous system and during convalescence from acute diseases. Certain drugs such as salicylates, calomel, and digitalis also cause polyuria.

Appearance. Normal urine has a yellow color ranging from straw to amber. It is usually clear and transparent when voided. On standing most urine samples develop a cloud or **nubecula** composed of nucleoprotein or mucoid. If the urine is alkaline, as it frequently is after a meal, it may be turbid because of the precipitation of calcium phosphate. Another cause of turbidity, on standing, is an alkalinity resulting from the decomposition of urea to form ammonia. Pathological urine may be turbid because of the presence of fat globules or pus cells. Pus cells indicate an infection in the urinary tract.

The color of normal urine is due to the pigments **urochrome, urobilin,** and **uroerythrin.** Urochrome, the principal pigment, is thought to be a combination of urobilin and urobilinogen with a peptide substance. It is a product of endogenous metabolism, and about the same amount is excreted in the urine daily in a normal individual. In disease the color of the urine may be abnormal. In fever it may be much darker than usual. A hemorrhage in the kidney or urinary tract will cause the urine to be red. In jaundice the urine may have a greenish color caused by the presence of bile pigments. In carbolic acid poisoning the urine may be black because of the presence of phenol derivatives. Many drugs and dyes used in coloring candy may appear in the urine, giving it a characteristic color.

Odor and Taste. Urine has a faint aromatic odor, said by some to be due to traces of volatile organic acids and by others to the presence of a compound of unknown structure called **urinod.** Many drugs and foods affect the odor. Eating asparagus gives the urine a characteristic asparagus odor. In acidosis the urine has a peculiar sweetish odor due to acetone. Old urine has an ammoniacal odor due to ammonia resulting from ammoniacal fermentation.

Normally urine has a salty taste because of the presence of sodium chloride. In diabetes the urine tastes sweet because of the presence of glucose. Before the days of the clinical laboratory, tasting the urine for sweetness was the practice in the diagnosis of diabetes.

Reaction. The reaction of urine may vary over a wide range, depending on several factors. As a rule it is acid, having a *p*H of about 6.0. On an ordinary diet a 24-hour sample of urine requires from 250 to 350 ml. of 0.1 *N* alkali to neutralize it to phenolphthalein. This is known as **titratable acidity.**

The reaction of the urine is related to the proportion of the various phosphates present. NaH_2PO_4 is acid, and Na_2HPO_4 is basic, in reaction. Which phosphates are excreted in the urine depends upon the reaction of the blood; that phosphate is liberated which will tend to maintain the blood at its normal *p*H. Shortly after a meal the urine is usually alkaline because of the concentration of acid, as HCl, in the stomach. During gastric digestion, on account of removal of HCl from the blood, the blood becomes more alkaline. This temporary increase in alkalinity is spoken of as the **alkaline tide** and is, in part, responsible for the feeling of well-being shortly after a hearty meal.

Foods have an important influence on the reaction of the urine. In general, protein foods increase the acidity of the urine because of the sulfur and phosphorus they contain. During metabolism they are oxidized to sulfuric and phosphoric acids, which tend to lower the alkali reserve of the blood. Vegetable foods usually produce an alkaline urine because of the alkaline ash which they give on oxidation. Even citrus fruits, although highly acid, produce an alkaline urine. The citric acid present in citrus fruits is oxidized in the body to carbon dioxide and water and is thus eliminated by the lungs. The alkaline ash of the fruit is responsible for the decrease in acidity of the urine.

Extremely high acidities are found in diabetic acidosis, as a result of the large quantities of β-hydroxybutyric and acetoacetic acids present. Urines with high acidity contain a large quantity of ammonium salts, indicating that ammonia has been used to neutralize acid instead of forming urea.

Specific Gravity. The specific gravity of urine normally ranges from 1.015 to 1.030. These values may vary considerably, depending on the water intake. Specific gravity is closely associated with the quantity of solids present. Roughly, the total solids in a liter of urine may be determined by multiplying the second and third decimal figures of the specific gravity by 2.6. Since Long suggested this method, the value 2.6 is known as **Long's coefficient.** Usually the specific gravity of urine is determined by means of a special type of hydrometer known as a **urinometer.** As a rule the specific gravity is inversely proportional to the volume of a 24-hour sample. However, this is not always true. One of the characteristics of a diabetic urine is a high specific gravity associated with a large volume. The high specific gravity is due to the sugar present. A knowledge of the volume of urine excreted in 24 hours, together with its specific gravity, gives a physician a good idea of how well the kidneys are functioning in removing the waste products of metabolism.

Normal Constituents of Urine. The composition of the urine varies from day to day, depending upon the diet. Table 11 gives the more important constituents, together wtih the amount of each in grams, which may be found in an average 24-hour sample of urine.

Organic Constituents of Normal Urine. *Urea.* The most important nitrogenous constituent of urine is urea. Normally the nitrogen of urine is present to the extent of 80 to 90 per cent in the form of urea. The urea content varies widely, depending upon the amount of protein in the diet. On a low-protein diet the amount of urea in the urine decreases. Under such conditions urea nitrogen may constitute only 60 per cent of the total nitrogen of the urine. Urea is a diuretic, which accounts for the fact that on a high-protein diet the volume of urine excreted is increased. Urea is best determined by converting it into ammonia by means of the enzyme **urease.** The ammonia formed is aerated into standard acid, and the excess of acid is titrated with standard alkali. This method, of course, also gives the ammonia already present in the urine. Therefore, to determine urea, ammonia must also be determined and the result subtracted from the total ammonia present after treatment with urease. The amount of urea normally present in a 24-hour sample of urine is about 30 grams.

TABLE 11

COMPOSITION OF A 24-HOUR SAMPLE OF NORMAL URINE

	GRAMS
Water	1200
Total solids	60
Urea	30
Ammonia	0.7
Uric acid	0.7
Creatinine	1.2
Hippuric acid	0.7
Amino acid nitrogen	0.2
Phenols	0.2
Chlorides as NaCl	12.0
Sodium	4.0
Potassium	2.0
Calcium	0.2
Magnesium	0.15
Total sulfates as SO_3	2.5
Inorganic sulfates as SO_3	2.0
Ethereal sulfates as SO_3	0.2
Unoxidized sulfur as SO_3	0.3
Phosphates as P_2O_5	2.5

Uric Acid. Uric acid is one of the important nitrogenous constituents of the urine. It is the end product of purine metabolism in man, the anthropoid ape, and the Dalmatian coach hound. The average amount of uric acid in a 24-hour sample of urine is about 0.7 gram. This value varies considerably, depending upon the diet. On a diet rich in nucleoprotein the value may be much higher. On a purine-free diet there is still some uric acid in the urine. This uric acid comes from the breakdown of nuclear material in the body.

Uric acid is usually present in the urine as urates. If a sample of urine is acidified, uric acid will be set free from the urates and on standing will show a sediment of red uric acid crystals. Frequently uric acid will crystallize from normal urine on standing, which may alarm the person from whom the sample came, although there is no cause for alarm. Pure uric acid crystals are white; the red color of the crystals found in the urine is due to urinary pigments incorporated in the crystals.

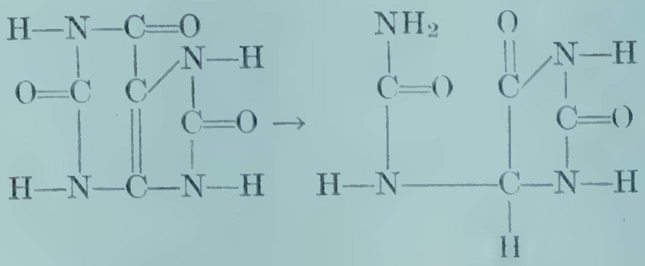
Uric acid is a mild reducing agent; it will reduce Fehling's solution. This fact should be considered in testing urine for sugar. A slight reduction of Fehling's solution in a sugar test may be due to uric acid. Uric acid will not reduce the bismuth subnitrate of Nylander's reagent; hence this reagent is useful when doubtful tests for sugar are obtained.

In disease the uric acid content of the urine may vary. In leukemia and during convalescence from pneumonia it is high because of the disintegration of nuclear material. In gout, uric acid is not eliminated normally by the kidney, with the result that there is less in the urine than normal and more in the blood. Preceding an attack uric acid output decreases but rises during the attack. Certain drugs, used in the treatment of gout, such as cinchophen and salicylates increase the excretion of uric acid. Under certain conditions uric acid crystallizes in the kidneys, forming stones. Alkali salts and base-forming foods tend to dissolve these stones by rendering the urine alkaline.

In birds and reptiles uric acid is the main nitrogenous constituent of the urine, taking the place of urea in man. Both birds and reptiles are hatched from eggs. During embryonic development the waste products of metabolism of the embryo must be removed. In the embryonic chick uric acid accumulates in a sack outside the body in the form of solid urate crystals. In mammals the metabolic products of the embryo are taken care of by the mother. Hence it is desirable to have a very diffusible end product of protein metabolism in mammals, and urea serves this purpose well.

Another explanation of why uric acid is the end product of protein metabolism in birds and reptiles is that uric acid is not a diuretic; that is, it is not a substance which stimulates the kidneys. In birds very little liquid urine is passed; the urine is little more than a mass of moist uric acid crystals. For this reason birds are relieved of the necessity of carrying large quantities of water for the purpose of making urine. This is a decided advantage, for extra weight would make it more difficult to fly. A low water requirement for reptiles makes it possible for them to live in arid regions, where the supply of water is scarce.

There are only traces of allantoin in human urine. In mammals other than man, the anthropoid ape, and the Dalmatian coach hound, uric acid is converted into **allantoin** before being excreted. Thus allantoin is the end product of purine metabolism in most mammals. Allantoin is found in the urine of fly maggots. Maggots have been used in wounds to clear up infections, the allantoin of their urine acting as an antiseptic.



Uric acid Allantoin

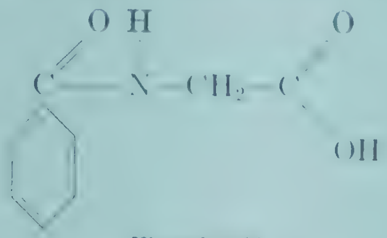
Creatinine. Creatinine, the anhydride of creatine, is present in the urine in quite constant amounts for a given individual. A 24-hour sample of urine from an average adult contains from 1 to 1.25 grams of creatinine. The milligrams of creatinine excreted in 24 hours per kilogram of body weight is known as the **creatinine coefficient.** It is higher for men than for women and children and is apparently related to muscular development. If creatinine is taken in the food, the amount in the urine increases. Long-continued feeding of creatine increases the creatinine content of the urine, an indication that creatine is converted into creatinine in the body. It is thought that the creatinine of the urine originates mainly from the creatine of the muscles, although some undoubtedly is derived from the creatine present in such foods as meat. It is thus evident that there should be a relationship between the amount of creatinine excreted and the amount of muscle tissue present in the body.

There is very little, if any, creatine in the urine of normal men. A small amount is found in the urine of children and in women during pregnancy. During fasting the amount increases.

Variations in the excretion of creatinine in disease have been noted, but as yet creatinine determinations on urine have been of little value in the diagnosis of disease. On the other hand, creatinine determinations on the blood are of great value in determining the severity of certain kidney disorders. A high blood-creatinine value indicates a very serious kidney condition because creatinine is normally very easily eliminated.

Creatinine gives a deep yellow color with alkaline picrate solution. This reaction is the basis of a colorimetric method for the determination of creatinine in urine and blood. Creatine is easily converted into creatinine by heating with hydrochloric acid. After conversion into creatinine, creatine may also be determined by the same color reaction.

Hippuric Acid. Hippuric acid is a combination of benzoic acid and glycine. It is present normally to the extent of about 0.7 gram in a 24-hour sample of urine. It results from benzoic acid, ingested as such or



Hippuric acid

arising from compounds in the food containing aromatic nuclei. Benzoic acid is detoxicated in the animal body by conjugating with glycine to form hippuric acid. The glycine either comes from the protein of the food or may be synthesized by the body. Benzoic acid is of more frequent occurrence in vegetable than in animal foods. Prunes and cranberries are especially rich in benzoic acid. Hippuric acid gets its name because of its occurrence in horse urine. The vegetable diet of the horse is responsible for the high concentration of hippuric acid in its urine.

Inorganic Constituents of Normal Urine. *Ammonia.* Ammonia is another end product of protein metabolism found in the urine. Normally about 0.7 gram of ammonia is present in a 24-hour sample of urine, which makes up about 4 per cent of the total nitrogen of the urine. It is usually present as the salts of hydrochloric, phosphoric, and sulfuric acids. According to Nash and Benedict and other investigators, the ammonia of the urine originates in the kidney. The main precursor of this ammonia appears to be the amino acids of the blood. According to Van Slyke the amide of glutamic acid, glutamine, is the main source of urinary ammonia.

In acidosis, during which large quantities of β-hydroxybutyric and acetoacetic acids are formed, they are eliminated partly as ammonium salts, and therefore in acidosis the excretion of ammonia is increased. Acid-forming foods, such as the proteins, increase the ammonia output; foods with an alkaline residue decrease it. Thus ammonia appears to be an important neutralizer of acids in the body.

Ammonia in the urine may be determined by making a sample alkaline, aerating the ammonia into standard acid, and titrating the excess acid with standard alkali. In determining total acidity of the urine, it is customary to add to the titratable acidity the acidity equivalent to the ammonia present. The resulting value gives a better indication of acid production by the body than titratable acidity alone, because the ammonia present is a measure of the potential acid neutralized.

Chlorides. The inorganic constituents of the urine are mainly the sodium, potassium, ammonium, calcium, and magnesium salts of hydrochloric, sulfuric, phosphoric, and carbonic acids. Of the salts present, sodium chloride predominates. A 24-hour sample of normal urine contains from 10 to 15 grams of chloride expressed as sodium chloride. This value, of course, varies considerably, depending upon the amount of salt in the diet. If sodium chloride is carefully eliminated from the diet, the amount of chloride in the urine can be reduced to a very low value. It is interesting to note that under these conditions the chloride content of the blood remains constant for a considerable period of time. If sodium chloride is withheld from the diet long enough, the chloride concentration in the blood gradually decreases until finally death results. Death is apparently due to a loss of sodium rather than of chlorine, because potassium chloride in the diet will not replace the sodium salt.

Frequently in nephritis chloride excretion may be diminished, with a resulting increase of chlorides in the blood. Under such conditions it is necessary to restrict carefully the amount of salt in the diet.

Phosphates. The phosphates occur in the urine in three forms: **alkaline phosphates,** which are the various sodium, potassium, and ammonium phosphates; **earthy phosphates,** which are the phosphates of calcium and magnesium; and **organic phosphorus,** such as that found in phospholipids, phosphoproteins, nucleoproteins, and nucleotides. The last-named form is present in only small amounts.

The amount of phosphate in the urine varies considerably with the diet. Normally a 24-hour sample of urine contains about 2.5 grams of phosphate expressed as P_2O_5. As a rule the alkaline phosphates are present in about twice the concentration of the earthy phosphates. Actually the proportion of alkaline to earthy phosphates in the urine is unimportant, the amount of earthy phosphate being simply an indication of the amounts of calcium and magnesium present in the urine. Under normal conditions the phosphates are present as the mono- and dihydrogen salts. The acidity of the urine is due mainly to the dihydrogen phosphates present.

The phosphates of the urine come mainly from the food. The phosphoproteins, such as casein, nucleoprotein, and phospholipids, all give rise to phosphoric acid in the body. Phosphates as such in the food contribute to the phosphate in the urine. Some of the phosphate in the urine arises from the phosphorus-containing compounds of the tissues.

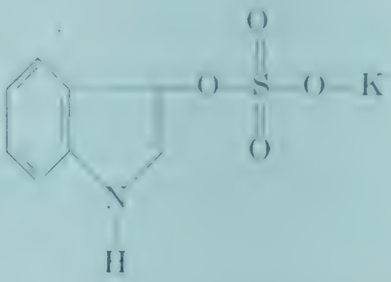
In certain diseases, especially those of the bone, such as rickets, the amount of phosphate in the urine increases.

During the later stages of pregnancy, when the fetal bones are being formed, the amount of phosphate in the urine is decreased.

From 35 to 50 per cent of the phosphate eliminated from the body appears in the feces, mainly as tricalcium phosphate. Many of the phosphates in the food are converted in the intestine, which is alkaline, into tricalcium phosphate, which is insoluble and is not absorbed. Some absorbed phosphate is eliminated directly into the intestine.

Sulfates. Sulfur is found in the urine in three forms: **inorganic sulfates, ethereal sulfates,** and **unoxidized** or **neutral sulfur.** The total sulfur, expressed as SO_3, in a 24-hour sample of urine usually amounts to about 2.5 grams but varies with the diet. Most of the sulfur comes from the sulfur-containing amino acids in the protein of the food. During the metabolism of these amino acids, most of the sulfur is oxidized to sulfate, so that the amount of sulfate in the urine varies with the amount of protein in the diet.

About 80 per cent of the total sulfur of the urine is in the form of inorganic sulfates, mainly the calcium, magnesium, ammonium, sodium, and potassium salts of sulfuric acid. About 10 per cent of the sulfur in the urine is in the form of sulfates conjugated with organic compounds like indole, skatole, phenol, and cresol. These sulfates are known as ethereal sulfates. Their formation is undoubtedly an example of protective synthesis, by means of which the body detoxicates noxious substances. Perhaps the most interesting of the ethereal sulfates is **indican,** which is indoxyl potassium sulfate. This compound should not be confused with the glucoside, indican.



Indican

When protein foods containing tryptophan putrefy in the intestine, indoxyl is formed, which is converted into indican in the body and eliminated in the urine as such. If a sample of urine is treated with **Obermayer's reagent,** which is $FeCl_3$ dissolved in HCl, the indican is hydrolyzed and oxidized to indigo blue, which may be extracted with chloroform. The chloroform takes on a blue color, the intensity of which depends upon the amount of indican present in the urine. This test has been made the basis of a colorimetric method for the determination of indican, which has been widely used as a test for intestinal putrefaction.

About 10 per cent of the total sulfur of the urine is present in the unoxidized form. Compounds included in this group are cystine, taurine, ethyl sulfide, hydrogen sulfide, and thiocyanates. The amount of sulfur present in the unoxidized form is rather constant from day to day and is not influenced greatly by the amount of protein ingested. For this reason it has been said to arise from the breakdown of tissue protein.

Carbonates. Carbonates are present in the urine in the form of bicarbonates, the amount being closely related to the diet. On an acid-producing diet the amount of bicarbonate in the urine is small; on a base-forming diet it may be considerable. Alkalinity of urine results largely from the presence of bicarbonates. The turbidity of alkaline urines is due in part to the presence of the bicarbonates of calcium and magnesium.

Pathological Constituents of the Urine. *Glucose.* An examination of the urine for certain abnormal constituents is often of great value to a physician in detecting diseased conditions. In the routine examination of urine a test for glucose is always made. Normal urine contains a trace of glucose but not sufficient to give a positive test with Fehling's or Benedict's solutions. The presence of glucose in the urine in appreciable quantities may be an indication of pancreatic diabetes. In interpreting a positive test for glucose in the urine, however, it should always be borne in mind that glucose may be present in the urine of persons who do not have pancreatic diabetes. If a large quantity of sugar has been eaten just previous to the test, it is possible for the urine to contain sugar. In such a case the concentration of sugar in the blood increases to the point where the sugar threshold is exceeded. Normally the concentration of glucose in the blood at the sugar threshold is from 160 to 180 mg. per 100 ml. of blood. In some individuals the sugar threshold is much lower than normal; in them glucose may be a constant constituent of the urine.

During pregnancy glucose may appear in the urine even though blood-sugar values are normal. During lactation lactose may be present in the urine. Lactose, being a reducing sugar, may be mistaken for glucose. Lactose can be distinguished from glucose by its formation of a typical osazone, by its response to the mucic acid test, and by the difficulty with which it is fermented by common bread yeast. A sample of urine containing lactose will retain its reducing power after treatment with yeast; one containing glucose will not. The presence of lactose in the urine has no pathological significance.

Under certain conditions pentoses, galactose, and fructose have been found in the urine. Pentoses may appear after eating large quantities of fruits such as plums, grapes, and cherries. Some persons normally have pentoses in the urine, the origin of which is unknown. Galactose has been found in the urine of nursing infants. Fructosuria is a rare condition, and its cause has not been discovered.

Certain drugs such as aspirin combine with glucuronic acid to form compounds that are excreted in the urine. Since glucuronic acid reduces Fehling's solution the presence of these compounds in the urine may give a false test for glucose.

A diabetic urine is usually characterized by a strong reducing power, a high specific gravity due to the glucose present, a large volume, and a light color. It is always advisable to determine the concentration of sugar in the blood whenever sugar is found in the urine. A high sugar value on a sample of blood taken before a meal is good evidence that the patient has pancreatic diabetes.

Albumin. The condition in which protein is found in the urine is known as **proteinuria** or **albuminuria.** Albumin is usually the main protein present although appreciable amounts of globulins may occur.

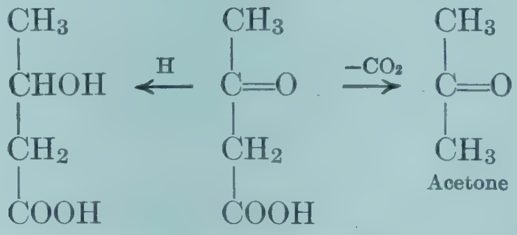
If the protein comes from the kidneys, being excreted from the blood, the condition is known as **renal** or **true albuminuria**; if it enters the urine from some point below the kidney, the condition is known as **accidental** or **false albuminuria.** Renal albuminuria is the more serious type and is an indication of a kidney disease known as **nephritis.** A normal person may have albumin in the urine as a result of exposure, violent exercise, or excitement. Some people show albumin in the urine after they have been standing for some time. This is known as **orthostatic albuminuria** and is apparently not serious.

Many tests are available for detecting the presence of protein in the urine. A simple, common one is the **heat-coagulation test,** in which the upper layer of urine in a test tube is heated over a free flame to boiling. A clouding may be due to albumin or to a precipitation of earthy phosphates. On acidifying with dilute acetic acid the phosphates will dissolve, whereas albumin will continue to cloud the sample.

Albumin in the urine is an indication of impaired kidney function. Several methods are available for determining the extent of this impairment. One method is known as the **phenolsulfonphthalein test** for kidney function. In this test a known quantity of this dye in a sterile solution is injected into the muscle. This dye is excreted only by the kidney. At intervals of 1 and 2 hours after the injection the bladder is emptied, and the amounts of dye present in each sample of urine are determined colorimetrically. These amounts are a measure of kidney efficiency. At least 40 per cent of the dye should be eliminated in the first hour and 60 per cent in 2 hours.

Another method of estimating kidney efficiency is to analyze the blood for waste products of metabolism normally found in the urine. If the kidneys are not functioning properly, the concentration of these waste products increases in the blood. Nonprotein nitrogen, urea nitrogen, uric acid, and creatinine are commonly determined for this purpose.

Acetone Bodies. The acetone bodies are β-hydroxybutyric acid, acetoacetic acid, and acetone. Their relationship is indicated by the following:



$$CH_3CHOHCH_2COOH \xleftarrow{H} CH_3COCH_2COOH \xrightarrow{-CO_2} CH_3COCH_3$$

β-Hydroxybutyric acid Acetoacetic acid Acetone

The acetone bodies originate from the oxidation of fatty acids, in which acetoacetic acid is one of the intermediate products. Normally acetoacetic acid is oxidized to carbon dioxide and water; under certain conditions, however, a reduction may take place with the formation of β-hydroxybutyric acid, or carbon dioxide may be removed, leaving acetone. The accumulation of acetoacetic and β-hydroxybutyric acids in the blood reduces its alkali reserve, producing a condition of acidosis. In diabetes and during starvation acetone bodies are commonly present in the urine.

Normal urine contains traces of acetone bodies. From 20 to 30 mg. of β-hydroxybutyric acid may be present, constituting the greatest part of the total acetone bodies. In severe acidosis there may be 6 grams of acetone plus acetoacetic acid and 100 grams of β-hydroxybutyric acid in a 24-hour sample of urine.

In a routine examination of urine acetone is usually tested for. If acetone is present, β-hydroxybutyric and acetoacetic acids are assumed to be present. A very simple test for acetone may be made by adding to some urine in a test tube a few drops of sodium nitroprusside solution and rendering alkaline with KOH. A deep red color, which may be due to creatinine or to acetone, develops. If due to acetone, the color remains after neutralizing with acetic acid; if due to creatinine, it disappears on adding the acid.

Blood. When blood appears in the urine, red cells may be present or only hemoglobin. The first condition is called **hematuria,** and the second **hemoglobinuria.** In hematuria, red cells can usually be detected in a urinary sediment by a microscopic examination. The presence of red cells indicates a hemorrhage in the kidney or the urinary tract. Hemoglobinuria is due to hemolysis, in which the red cells rupture and the hemoglobin is discharged and finally excreted in the urine. This occurs in several diseases. Blood may be detected in the urine by the **benzidine** or **guaiac tests,** in which benzidine or guaiac solutions are added to the urine, together with a fed drops of hydrogen peroxide. A blue color indicates the presence of blood.

Microscopic Examination. Many interesting things may be learned from a microscopic examination of a urinary sediment. (See Fig. 35.)

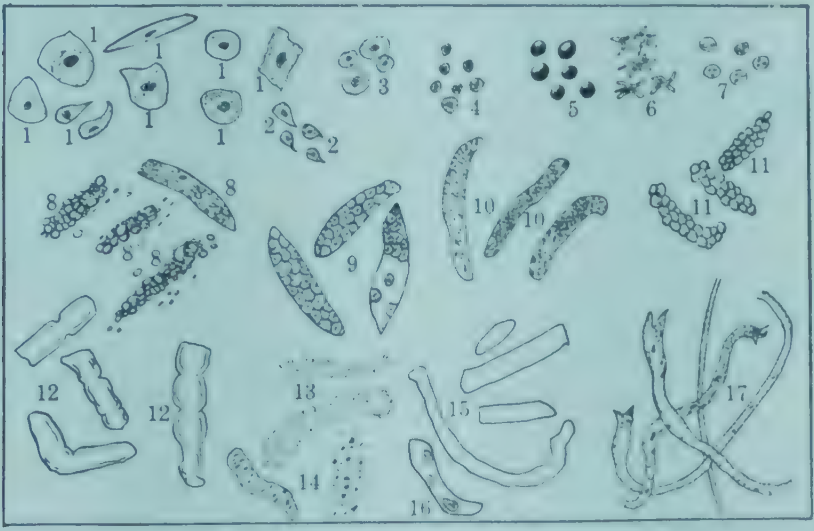


FIG. 35. Cells and casts found in urinary sediments: (1) squamous epithelial cells from the bladder; (2) superficial pelvic cells; (3) cells from neck of bladder; (4) renal cells; (5) pus cells (normal); (6) pus cells (ameboid form); (7) pus cells (nuclei made visible with acetic acid); (8) fatty casts; (9) epithelial casts; (10) blood casts; (11) pus casts; (12) waxy casts; (13) finely granular casts; (14) coarsely granular casts; (15) hyaline casts; (16) hyaline cast containing renal cells; (17) cylindroids.

The sediment may contain crystals of various kinds, such as phosphates, oxalates, urates, and uric acid, and amino acids, such as cystine. Epithelial cells from the lining of various regions of the urinary tract may be present. Of special significance are pus cells and casts. An occasional pus cell may be found in normal urine. Their presence in large numbers, however, indicates an infection in the kidney or urinary tract. A condition of pus in the urine is known as **pyuria.** Pus cells are white blood cells and can be distinguished from other cells by their nuclei and peculiar granular appearance. Urine containing pus usually gives a positive test for albumin.

Under certain conditions the uriniferous tubules of the kidney may become filled with material of various kinds. These plugs may finally be discharged into the urine and be present in the urinary sediment. They appear under the microscope as cylinders with rounded ends and are called **casts.** Some casts are hyaline, others are granular, and still others are filled with pus cells, epithelial cells, or fat globules. Each type of cast has its own particular significance. The presence of casts in the urine is of great pathological significance. The presence of albumin, together with casts, indicates a more serious condition than the presence of casts alone.

Frequently in a urinary sediment bodies called **cylindroids,** which are often mistaken for casts but which have no pathological significance, are observed. They may be distinguished from casts as they are usually longer and narrower, they tend to be flat rather than round, and they frequently have branching ends.

REVIEW QUESTIONS

1. Name the excretory organs of the body.
2. Describe the structure of the kidney.
3. Give a theory explaining the formation of the urine.
4. What is the relation of blood pressure and blood colloids to urine formation?
5. What is meant by a threshold substance?
6. What is the normal volume of a 24-hour sample of urine?
7. What is meant by a diuretic, anuria, polyuria, and oliguria?
8. To what is the color of urine due?
9. Under what conditions is the urine cloudy?
10. What is the odor and taste of normal urine? Under what conditions may urine have an abnormal odor and taste?
11. What is the reaction (pH) of normal urine? Under what conditions does the reaction of urine vary from normal?
12. What is the normal specific gravity of urine? What do high and low values indicate?
13. What is meant by Long's coefficient? Of what value is it?
14. Name the important organic constituents of the urine. How many grams of each are present in a normal 24-hour sample of urine?
15. Name the important inorganic constituents of the urine. Which one is normally present in the largest amount?
16. What is the origin of urinary ammonia?
17. Name three types of phosphorus found in the urine.
18. What is the origin of urinary phosphates?
19. Name three types of sulfur found in the urine.

20. What is the origin of urinary sulfates?

21. Describe a urinary test for intestinal putrefaction.

22. Name the pathological constituents of the urine and indicate the significance of each. How is each tested for?

23. Distinguish among true, false, and orthostatic albuminuria.

24. Describe the phenolsulfonphthalein test for kidney function.

25. What may be found in a microscopic examination of the urine?

26. What are casts? What is pyuria?

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20

Endocrine Organs

Many organs in the body produce secretions which pass directly into the blood stream. Because these secretions are not carried from the organs by ducts, the organs are sometimes called the **ductless glands** or, more commonly, the **endocrine organs.** The secretions of the endocrine organs have marked physiological properties. The active constituents of these secretions are called **hormones.** These hormones appear to be body regulators and are essential for the proper functioning of the body. In fact, many of the hormones are essential for life, and death will result if the organ producing them is removed.

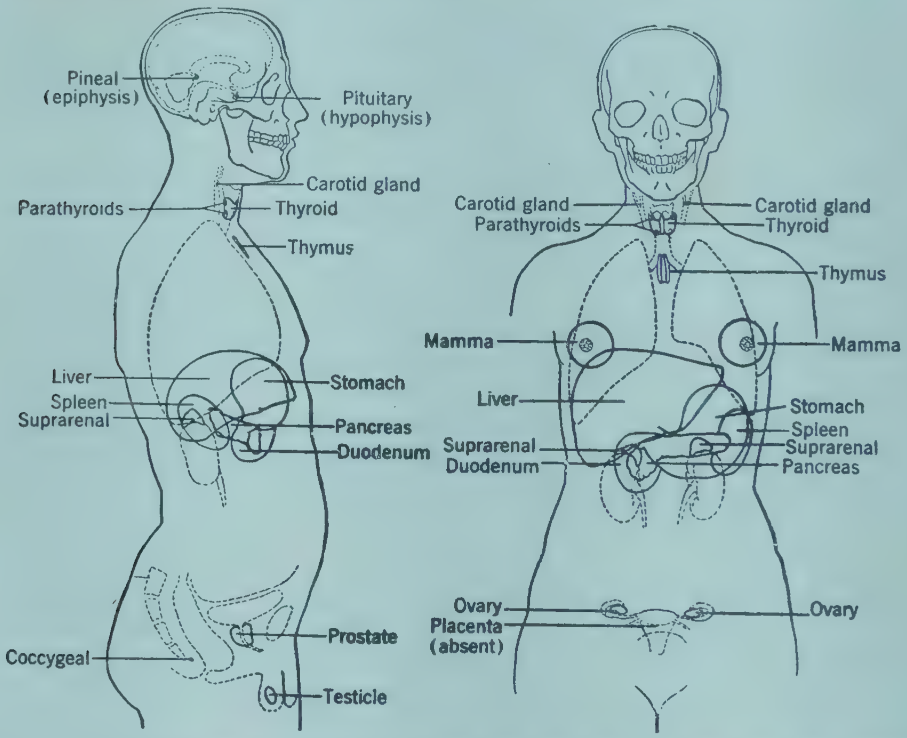
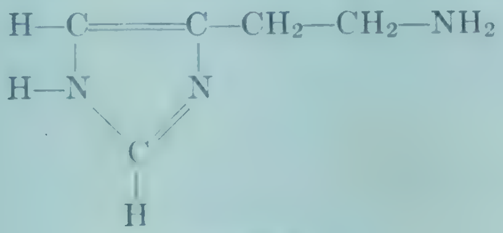

Pineal (epiphysis)
Pituitary (hypophysis)
Carotid gland
Parathyroids
Thyroid
Thymus
Liver
Spleen
Suprarenal
Stomach
Pancreas
Duodenum
Prostate
Coccygeal
Testicle
Carotid gland
Parathyroids
Carotid gland
Thyroid
Thymus
Mamma
Mamma
Liver
Stomach
Spleen
Suprarenal
Pancreas
Suprarenal
Duodenum
Ovary
Placenta (absent)
Ovary

Fig. 36. Diagrammatic charts showing the location of the various endocrine organs. From *Endocrinology and Metabolism* by Lewellys F. Barker, M.D. Used by permission of D. Appleton-Century Co.

From the critical standpoint it might be said that every organ or tissue of the body produces substances that pass directly into the blood stream and that have an effect on other parts of the body. For example, carbon dioxide stimulates respiration. From this viewpoint all tissues might be looked upon as endocrine organs. However, in this chapter the term endocrine organ will be used in its narrower sense and will refer only to organs that produce special compounds of a hormonal nature. The most important of these organs are the organs of the digestive tract (the **stomach** and the **intestines**), the **pancreas**, the **thyroid**, the **parathyroids**, the **adrenals**, the sex organs (the **ovaries** and **testes**), and the **pituitary**. (See Fig. 36.) Strictly speaking hormones are substances that stimulate various organs of the body. Some endocrine secretions have inhibitory effects and are called **chalones.**

The Digestive Tract

Stomach. In studying digestion it will be recalled that several hormones were mentioned which are associated with the production of the digestive juices. The secretion of gastric juice by the stomach is said to be due, at least in part, to a hormone, found in the mucosa of the pylorus, called **gastrin.** If extracts of pyloric mucosa are injected into an animal, there is an increase in gastric secretion. It is believed by some that the active principle of gastrin is **histamine.** Histamine is sometimes injected to stimulate gastric secretion when a laboratory examination of gastric juice is required for diagnostic purposes.



Histamine

Intestines. Five hormones have been described that are present in the intestinal wall, namely **enterocrinin**, **secretin**, **pancreozymin**, **cholecystokinin**, and **enterogastrone.**

Enterocrinin, a hormone prepared from intestinal mucosa by Nasset, Pierce, and Murlin, stimulates the intestinal glands to produce intestinal juice.

Secretin stimulates the pancreas to secrete pancreatic juice. It also regulates the volume of juice secreted. The presence of acid in the duodenum stimulates the production of secretin. That the stimulation of the pancreas is due to a hormone is indicated by the fact that, if the circulations of two dogs are crossed and acid is placed in the duodenum of one dog, pancreatic juice will flow in both dogs. This experiment indicates that the stimulating principle is carried in the blood. Also, an acid extract of the duodenum, injected into an animal, will stimulate the flow of pancreatic juice. The chemical nature of secretin is not known.

Pancreozymin stimulates the production of enzymes by the pancreas. It has no effect upon the volume of pancreatic juice produced.

Cholecystokinin, a hormone closely associated with secretin, causes a contraction and evacuation of the gall bladder. It is produced under conditions similar to those that produce secretin, and its presence has been proved by similar methods. Its chemical nature is not known.

Enterogastrone is an intestinal hormone that inhibits gastric secretion and motility. It is produced when undigested fat is present in the intestine. Its chemical nature is not known.

The Pancreas

In Chapter 12 on carbohydrate metabolism it was pointed out that the pancreas produces an internal secretion called **insulin,** whose function is to regulate the oxidation of sugar in the animal body. In the absence of sufficient insulin the animal develops the disease known as diabetes mellitus.

Effects of Removal of the Pancreas. In 1889 von Mering and Minkowski discovered that, if the pancreas is removed, an animal develops all the symptoms of human diabetes. The blood sugar rises, sugar appears in the urine, and there is a complete loss of ability to oxidize carbohydrate. The animal loses weight and is very thirsty, hungry, and weak. The volume of urine is greatly increased, and, besides sugar, the urine contains large quantities of acetone bodies. There is reduced resistance to infection. The wounds from the operation became infected and healed with difficulty. The animals died in the coma of acidosis in 4 to 6 weeks.

Further work to prove that the pancreas furnishes an internal secretion was done by Hédon, who operated on a dog and removed most of the pancreas; the remaining part he transferred to a position under the skin. As long as some of the pancreas remained in the body, even though it did not supply pancreatic juice to the intestine, no symptoms of diabetes appeared.

Structure of the Pancreas. The pancreas is composed of two types of tissue. One, called **acinar,** produces the pancreatic juice, which flows through the pancreatic ducts to the intestine where it performs its digestive functions. The other, called **islet tissue,** which has no communication with ducts, is now believed to produce the hormone of the pancreas which passes directly into the blood stream. Langerhans, in examining stained sections of pancreas, first noticed groups of cells which appeared like little islets. These groups of cells are now known as **islands of Langerhans.** Since the pancreatic hormone is believed to be produced by the islet tissue, it has been called insulin.

Insulin. Many attempts were made to prepare extracts of pancreas which could be used in the treatment of diabetes, but little success was achieved until 1921, when a group of workers in Toronto, Canada, headed by Banting, Best, Collip, Macleod, and Noble, solved the problem. As a result of their work insulin is now produced in quantity and has proved a valuable aid in the control of diabetes.

Although insulin has been prepared in a very pure form, its chemical structure is not known. Abel prepared crystalline insulin, which is protein in nature. Jensen believes that the physiological activity of insulin is due to a combination of glutamine and cystine in the molecule. It has been suggested that the S—S linkage of cystine is the important chemical structure. Another view is that tyrosine present in the molecule is related to its physiological activity. Cystalline insulin contains small amounts of zinc.

Because it is a protein, insulin cannot be taken with benefit by mouth. The proteolytic enzymes of the digestive tract hydrolyze it so that by the time it is absorbed it is no longer insulin. For the treatment of diabetes it is injected into the muscles. For medicinal purposes the strength of an insulin solution is expressed in terms of insulin units. A unit of insulin is an amount of insulin equivalent in activity to $^{1}/_{22}$ mg. of a standard preparation of crystalline zinc insulin kept by the National Institute of Medical Research in London.

One of the difficulties encountered in using insulin is that it is very rapid in its action of lowering blood sugar, and its effect soon wears off. There is great danger that the blood sugar may be reduced too much, with the result that the patient goes into what is known as insulin shock. Also the blood-sugar level varies considerably from hour to hour during the day. For best results pure insulin must be given before each meal. A great advance in insulin preparation was made by Hagedorn, who combined insulin with protamine. This combination, when injected, is absorbed slowly, so that a day's requirement may be injected at one time, and the blood-sugar level remains quite constant during the period between injections. There is much less danger from insulin shock than with pure insulin. Another form of insulin which retains its activity even longer than protamine insulin is a combination of insulin with protamine and zinc. Still another product which has recently been introduced is a combination of insulin and globin. Globin insulin retains its activity longer than insulin but not so long as protamine zinc insulin.

The main function of insulin appears to be as an essential in the formation of glucose-6-phosphate, an important intermediate in carbohydrate metabolism. If this phosphate is not formed glucose is not oxidized and its storage as glycogen in the liver and muscles is prevented. In the absence of insulin, fat is mobilized in the fat depots and accumulates in the liver and blood. When carbohydrates are not oxidized, the body attempts to obtain its energy from the oxidation of fats and ketogenic amino acids, with the result that acetone bodies accumulate, giving rise to a condition of acidosis.

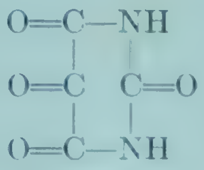
Hyperinsulinism. Although the more common pancreatic disorders involve an underproduction of insulin, it should be pointed out that under certain conditions the pancreas may produce too much insulin. This condition is known as hyperinsulinism. Individuals with this condition have low blood-sugar values and may develop symptoms of insulin shock. There is extreme weakness and a tendency to faint. The author knows of a young man who frequently fainted shortly after arising in the morning. Frequent examinations of his blood revealed glucose values of around 60 mg. per 100 ml. When he ate glucose before arising, he had no more fainting spells.

In certain types of insanity beneficial results have been noted by injecting sufficient insulin to lower the blood sugar to the point of shock.

Other Hormones Associated with the Control of Blood Sugar. From the foregoing discussion it might be concluded that insulin is the only hormone associated with the control of the blood-sugar level. This, however, is not the situation. Insulin is a blood-sugar depressor. Several hormones from other endocrine organs have the opposite effect. Among them may be mentioned **adrenaline, thyroxine,** and a **hormone of the pituitary.** If the pancreas is removed from a dog, he develops diabetes. If later the pituitary is removed, the diabetic symptoms are much subdued. It is believed that a hormone in the pituitary is antagonistic to insulin and that in health there is a balance between these two hormones. When insulin is lacking, the hormone of the pituitary elevates the blood sugar. When both are lacking, blood-sugar levels are more normal.

It is evident from what has been said that the control of the blood-sugar level is a complicated affair related to several of the endocrine organs. The pancreas, therefore, should not immediately be blamed for every variation from normal of blood-sugar values. It is quite possible that a departure from normal blood-sugar values may be due to a disturbance in one of the other endocrine organs associated with control of blood-sugar level.

Alloxan Diabetes. If alloxan is injected into an animal pancreatic diabetes results. Alloxan destroys the islet tissue of the pancreas thus interfering with the production of insulin. Alloxan is a pyrimidine and therefore is closely related, chemically, to the pyrimidines and purines of nucleic acid. There is no evidence to indicate that human diabetes may be caused by the production of alloxan in the body.

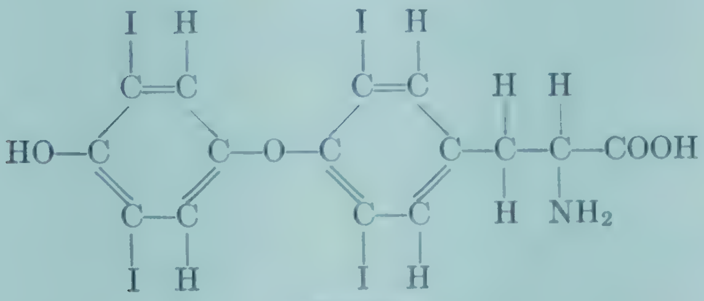


Alloxan

The Thyroid Gland

The thyroid is a small gland, weighing about 25 grams, located in the neck. Histologically it is made up of closed alveoli filled with a colloidal material which contains the active principle of the gland.

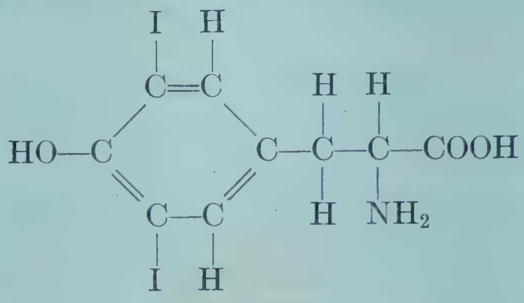
Thyroxine. The active principle of the thyroid gland was first isolated by Kendall, who called it thyroxine. Later Harington and Barger showed it to be a derivative of tyrosine and assigned to it the following formula:



Thyroxine

Thyroxine synthesized in the chemical laboratory has the same physiological properties as that obtained from thyroid glands. Thyroxine is found in the thyroid gland in a protein material called **thyroglobulin**, which is the principal constituent of the colloid of the gland. Some consider thyroglobulin to be the actual hormone since thyroxine is found only in small amounts in the free state in the body.

Another compound called **diiodotyrosine** is also found in thyroglobulin. Although it has no physiological activity it is thought to be the substance from which thyroxine is made in the thyroid gland.



Diiodotyrosine

Hypothyroidism. If the thyroid gland develops insufficiently in embryonic life, the child becomes a dwarf or **cretin.** The term cretin is derived from the fact that the condition is common on the island of Crete. A cretin grows slowly and has a low mentality; his hair is scanty and coarse, and his skin is thick and dry. This condition is often spoken of as **myxedema** because of the thick, dry skin. If the child is given thyroids to eat or thyroxine, he does not become a cretin but develops normally. This treatment, however, must be continued indefinitely to prevent the child from developing into a cretin.

If the gland is removed or if it becomes subnormal in activity in an adult, myxedema develops. The skin becomes thick and dry, the hair coarsens and falls out, and there is a disinclination to work, either physically or mentally. There is a tendency to put on weight. The metabolic rate is reduced, nitrogen metabolism is lowered, and body temperature is subnormal.

Hyperthyroidism. If the thyroid gland is too active, a condition known as **exophthalmic goiter** results. A common symptom of this disease is a bulging of the eyes, hence the name. (See Fig. 37.) The disease is also known as Graves' and as Basedow's disease. The symptoms of exophthalmic goiter are just the opposite of myxedema. The basal metabolic rate is high instead of low, nitrogen metabolism is increased instead of diminished, the hair is fine instead of coarse, the body temperature is above instead of below normal. The patient is nervous and irritable instead of sluggish; his mentality is above rather than below normal; and he is underweight rather than overweight. His heart beat is generally fast and irregular.

Usually but not always the thyroid gland is enlarged in exophthalmic goiter. This condition can be cured by removing part of the thyroid gland; the improvement in the patient produced by this procedure is remarkable.

Hyperthyroidism may also be treated by the use of drugs. Among

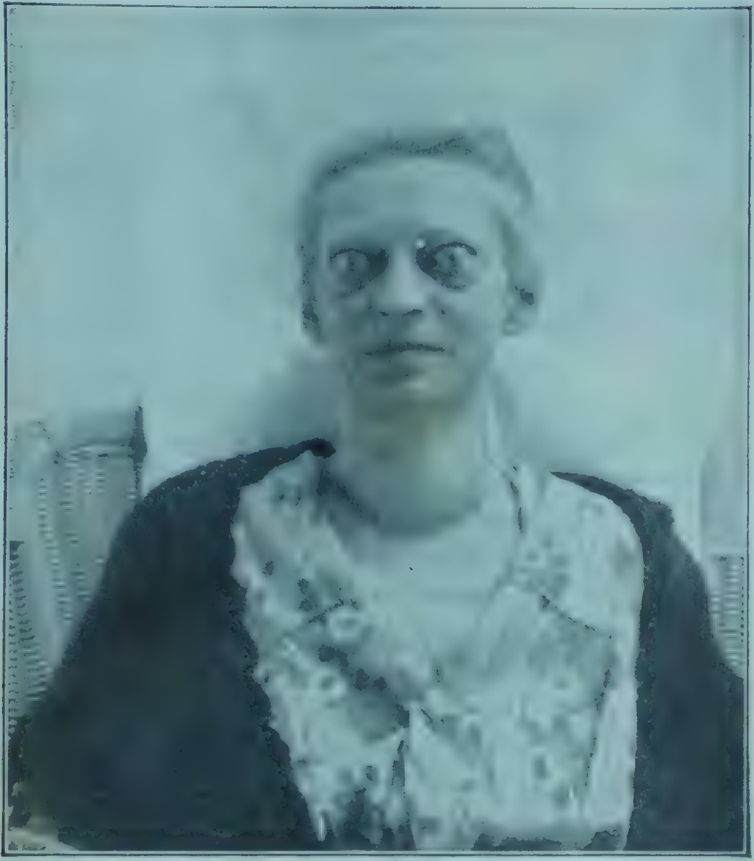
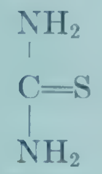


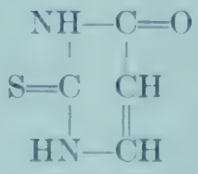
FIG. 37. Exophthalmic goiter. Courtesy of Drs. H. L. Foss and H. F. Hunt, Geisinger Memorial Hospital.

these are thiourea, thiouracil, propylthiouracil, and the sulfa drugs. These are thought to act by inhibiting either the uptake of iodine by the thyroid gland or the formation of thyroxine from diiodotyrosine.



$\mathrm{NH_2{-}C({=}S){-}NH_2}$

Thiourea



Thiouracil

Simple Goiter. The most common type of goiter is known as **simple or endemic goiter.** In this condition there is an enlargement of the thyroid gland, which is evinced by a swelling in the neck. In simple goiter the basal metabolic rate is normal or below normal. In other words, simple goiter is a condition of hypo- rather than hyperfunction of the thyroid gland. In fact, the enlargement of the thyroid may be looked upon as nature's attempt to produce the required amount of thyroid hormone. The increase in size of the gland is due to an increase in the amount of colloid present; hence this type of goiter is sometimes called **colloid goiter.** The administration of iodine in the form of iodides is beneficial. Many clinicians believe that simple goiter is the first stage of exophthalmic goiter.

Iodine and Its Relation to Goiter. Since the active principle of the thyroid gland is so rich in iodine, it appears that an adequate supply of this element in the food is necessary for the proper functioning of the gland. Iodine is found widely distributed in foods and water, but in many regions the amounts present are extremely small. Sea water is comparatively rich in iodine, and along the seacoast, where spray is carried inland, the soil contains the element. Drinking water and crops from these regions are richer in iodine than those from inland regions. Simple goiter is much less common in regions near the sea than in inland regions.

In the interior of the United States, especially around the Great Lakes and in the Northwest, goiter is very common. McClendon has shown that the incidence of goiter is closely associated with the amounts of iodine in the drinking water and in the crops grown in any particular locality. Lake Superior water is very low in iodine content. It has been estimated that a person would have to drink Lake Superior water for 2000 years to supply the iodine normally present in his body.

It has been found that supplying iodine in the form of sodium iodide in the diet reduces the incidence of simple goiter remarkably. Marine and Kimball conducted a now classical experiment on the school children in Akron, Ohio. They introduced a small quantity of sodium iodide into the drinking water of 2000 school children. They were given this water twice weekly for a month, and this treatment was repeated twice each year. After several years the group was examined for evidences of thyroid enlargement. Only 5 out of the 2000 showed symptoms of simple goiter. Of a similar group not receiving the sodium iodide 500 showed evidence of thyroid enlargement. According to these figures, 99 per cent of the incidence of simple goiter may be prevented by supplying iodine in the diet.

Perhaps the most practicable way of supplying iodine in the diet is by the use of iodized salt, which may be purchased at any grocery store. It has been said that the inclusion of an adequate supply of iodine in the diet from early childhood would eliminate the occurrence of simple goiter. In this connection it should be mentioned that an adequate supply of iodine is needed in the diet during pregnancy in order to insure freedom from thyroid disorders in the child. For individuals in whom thyroid abnormality is already present the use of iodized salt is questionable; it should be taken only upon the advice of a physician.

since in certain types of goiter the use of iodides is contra-indicated.

Iodine is essential not only for human beings but also for animals. In Michigan sheep raising was almost abandoned because of the difficulty in producing wool of good quality. Ewes bore few young, and many of these died soon after birth. Feeding iodized salt to the sheep remedied the condition.

From what has been said it is evident that goiter is a nutritional-deficiency disease. If the relation of iodine to goiter had not been known before the discovery of vitamins, it is very probable that iodine would now be known as the antigoiter vitamin.

The Parathyroid Glands

The parathyroid glands were discovered by Sandström in 1880. They are minute glands, weighing about 0.1 gram each, which are closely associated with the thyroid gland. They are usually four: two of them, embedded in the thyroid gland, are known as the internal parathyroids; the other two, lying close to and behind the thyroids, are known as the external parathyroids. Some animals have more than four parathyroid glands; the extra ones are scattered along the trachea and known as accessory parathyroids. Because they are so closely associated, it is difficult to remove the thyroids without also removing the parathyroids. Many deaths which have been attributed to the removal of the thyroid can be explained instead by the removal of the parathyroids along with the thyroids.

Effects of Parathyroid Removal. If the parathyroids are completely removed in a dog, very striking symptoms appear. A day or so after the operation the animal shows symptoms of intoxication and loses muscular control. As time passes, tremors appear which become more and more violent. There is a decided increase in body temperature, and respiration and heart action become more rapid. A bloody diarrhea is a common symptom. Finally, on the ninth or tenth day the animal dies in convulsions. An analysis of the blood shows a decided decrease in its calcium and an increase in its phosphorus content. Whereas normal blood serum contains about 10 mg. of calcium per 100 ml., after removal of the parathyroids the amount may be reduced to as little as 5 mg. This lowering of the blood calcium is undoubtedly the cause of the tetany which accompanies the removal of the parathyroids. Postmortem examination indicates degenerative changes in the kidney and intestinal mucosa, accompanied by intestinal hemorrhage.

The Parathyroid Hormone. That the parathyroid glands are endocrine organs has been shown by the work of Hanson and of Collip, who have isolated the parathyroid hormone from the glands. This hormone is called **parathormone** and is believed to be a protein. By means of his preparations Collip has been able to prevent tetany in dogs in which the parathyroids have been removed. Injection of the hormone into dogs already in tetany relieves the condition. The hormone also restores blood calcium and phosphorus to their normal levels. Given to normal animals, the hormone causes a marked rise in blood calcium and a fall in blood phosphorus. If parathormone is administered in excess and the blood-calcium values rise high enough, death ensues. The calcium entering the blood comes from the calcium in the bones.

An interesting disease, known as **von Recklinghausen's disease** of the bone, has been shown to be due to overactive parathyroids. In this disease blood-calcium values are high, phosphorus values are low, and the bones lose their mineral matter. The bones cause pain, they fracture easily, and may become deformed.

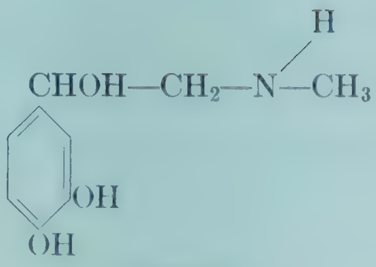
It is thus evident that the parathyroid glands produce a hormone which is closely associated with calcium and phosphorus metabolism. In this respect the parathyroid hormone resembles the antirachitic vitamin D in its action. Just what relationship exists between the two is unknown. Many clinicians believe that tetany in children may be due to subnormal parathyroid activity. Parathormone has been used with success in treating this condition.

The Adrenal Glands

The adrenal glands are small organs located at the upper end of each kidney. In an adult a single gland weighs about 3 grams. Each gland is composed of two parts, an internal region called the **medulla** and an external layer called the **cortex.** The medulla is characterized by being stained yellow with potassium dichromate. Tissues which take this yellow stain are called **chromaffine tissues;** their occurrence is not confined to the medulla of the adrenal glands.

Adrenaline. The staining of the medulla is due to the presence of the active principle which it produces. This active principle or hormone has been given the name **adrenaline** or **epinephrine.** Adrenaline is one of the hormones that have been isolated in a pure form and whose chemical structures are known. Abel first prepared adrenaline as the benzoyl derivative. Takamine first isolated it in the pure form. Aldrich also isolated the pure substance and determined its chemical structure. Adrenaline has been prepared synthetically.

Adrenaline, when injected into an animal, has marked physiological effects. It produces a decided increase in blood pressure and stimulates



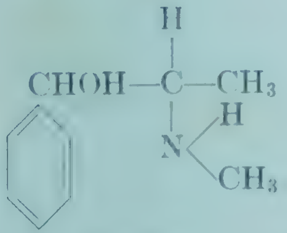
Adrenaline

the heart. For this reason it is used in medicine in cases of shock. It causes a contraction of the arterioles and therefore is of value in reducing hemorrhages. It is useful in asthma because it relaxes the bronchioles. Injected into the blood stream, it induces a rise in blood-sugar concentrations which may be sufficiently high to cause glucosuria. In cats it produces symptoms of fear, such as erection of hair and dilation of the pupils of the eye.

One theory of the function of adrenaline is that in times of danger it is poured into the blood, causing an increase in the blood-sugar concentration. This high concentration of sugar provides fuel for the muscles, supplying the individual with energy to carry him through the emergency.

Although adrenaline has many physiological activities, the adrenal medulla, which produces it, is not considered an organ essential for life. If the adrenal medullas are removed from an animal there are no noticeable effects.

Ephedrine. A compound very closely related to adrenaline, called ephedrine, is found in a Chinese plant, *Ephedra vulgaris*. Ephedrine is used extensively in medicine. Its action is more persistent than that of adrenaline. It is used widely as an ingredient of nasal sprays. In head colds it is very effective in relieving congestion and opens the air passages by shrinking the nasal membranes. Ephedrine has the following formula:



Ephedrine

Cortical Hormones. If the adrenal glands are removed, most animals die in about 10 days. For several days after the operation they appear normal. Finally symptoms of extreme exhaustion appear, and the animals die. The administration of adrenaline does not prevent death and apparently has no beneficial effect. Hence, if the adrenal glands produce a hormone essential to life, it must be something other than adrenaline.

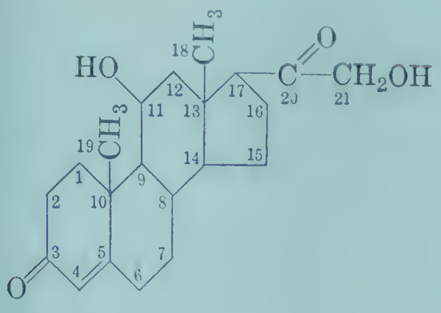
In 1885 Addison showed that a certain disease, recognized by a characteristic bronzing of the skin, anemia, low blood pressure, and lassitude, is associated with a degeneration, usually of a tuberculous nature, of the adrenal glands. This disease is now known as **Addison's disease.** For a long time it was thought that Addison's disease was due to the lack of some hormone originating in the adrenal glands. Adrenaline, the hormone of the medulla, has no effect in alleviating the symptoms of this disease. Work by Stewart, Swingle, Hartman, and others has shown that the vital hormone of the adrenal glands is located in the cortex. Extracts of the cortex have been made which have remarkable effects in prolonging the life of animals from which the adrenal glands have been removed and of human beings suffering from Addison's disease.

Physiologically Addison's disease appears to be associated with an abnormal metabolism of sodium. In this disease large quantities of sodium are excreted in the urine, resulting in a lowered concentration of sodium in the blood. Along with this condition there is an elevation in the blood potassium. Beneficial effects have been obtained by feeding diets high in sodium and low in potassium.

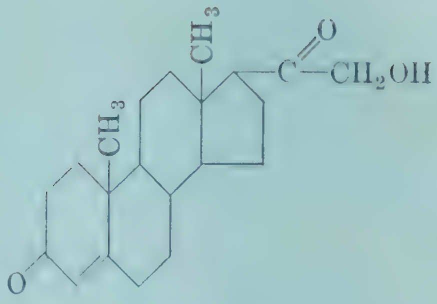
Among other physiological effects of lowered adrenal-cortex activity may be mentioned a decrease in kidney function, an interference with the proper utilization of carbohydrates, and a loss of water from the tissues.

The active principle of the adrenal cortex has been given the name of **cortin,** but it is now known that the activity of cortical extracts is not due to a single hormone but rather to a group of hormones. Kendall, Reichstein, and others have isolated twenty-eight compounds from the adrenal cortex which are all sterol derivatives. Of these only six have shown significant physiological activity. These are **corticosterone, desoxycorticosterone, 11-dehydrocorticosterone, 11-dehydro-17-hydroxycorticosterone,** also called cortisone and Compound E, **17-hydroxycorticosterone,** also called Compound F, and **adrenosterone.**

The most active derivative in treating Addison's disease is **desoxycorticosterone,** which differs from corticosterone by having the OH group tied to the ring replaced by H. Of the various derivatives of corticosterone isolated from the adrenal cortex none will completely replace cortin in treating Addison's disease. Although desoxycorticosterone causes the retention of sodium and water, it has little effect on carbohydrate metabolism.


HO
18
CH3
O
C
CH2OH
19
CH3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 20 21
O

Corticosterone


CH3
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C
CH2OH
CH3
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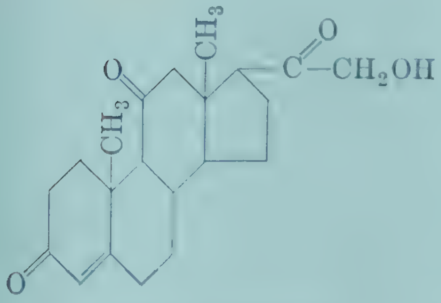
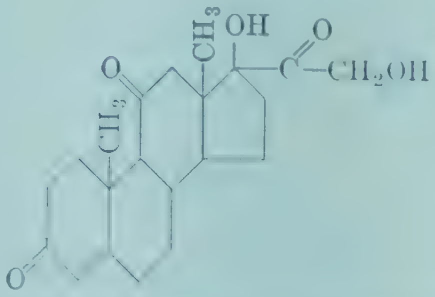
Desoxycorticosterone


CH3
O
C
CH2OH
O
CH3
O

11-Dehydrocorticosterone


CH3
OH
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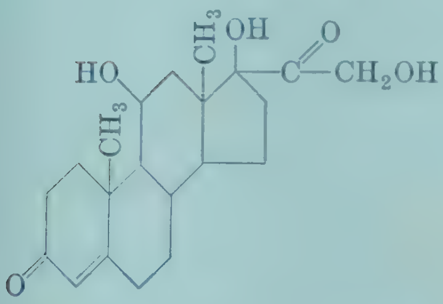
CH2OH
O
CH3
O

11-Dehydro-17-hydroxycorticosterone (Compound E or cortisone)


CH3
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CH2OH
HO
CH3
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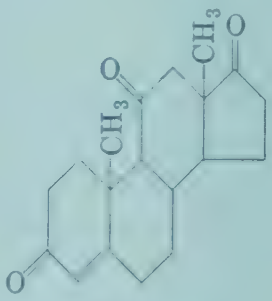
17-Hydroxycorticosterone (Compound F)



Adrenosterone

Cortisone. During World War II there were rumors that German airplane pilots were being given adrenal hormones to enable them to fly at high altitudes. Because of this the National Research Council inaugurated an extensive study of the hormones of the adrenal cortex. Although none of the results proved to be of value in aviation, other startling results were obtained. Among the compounds studied was cortisone, which was synthesized in the Merck and Company, Inc., laboratories by Sarett. The clinical work was done by Hench, Kendall, Slocumb, and Polley at the Mayo Clinic. Dr. Hench, who is a specialist in rheumatic diseases, was of the opinion that some hormone of the adrenal cortex might be helpful in such conditions as rheumatoid arthritis and rheumatic fever. He therefore administered cortisone to patients suffering from these diseases. The results were dramatic. Patients bedridden with rheumatoid arthritis were out of bed after a few days of treatment. In a week or two most of the pain and swelling in the joints was gone. When cortisone was withdrawn most patients reverted to their former condition. Thus cortisone must be given continuously to patients with rheumatoid arthritis. It is most useful in active cases and is of little value in old so-called burned-out cases.

Cortisone has proved of value in many diseases besides rheumatoid arthritis and rheumatic fever. Among these may be mentioned Addison's disease, asthma, hay fever, and inflammatory eye diseases. The discovery of cortisone and its importance in medicine will go down in medical history with the discovery of insulin, the control of pernicious anemia, and the development of the sulfa drugs and antibiotics. For their contributions in this field, Drs. Hench, Kendall, and Reichstein were awarded the Nobel Prize in medicine in 1950.

The antirheumatic properties of steroid compounds are related to their chemical structure. There must be a ketone group at carbon 3, a double bond between carbons 4 and 5, either a ketone or an hydroxyl group at carbon 11, hydroxyl groups at carbons 17 and 21, and a ketone group at carbon 20. Only cortisone and Compound F meet these requirements. In this connection it should be mentioned that Compound F has now been synthesized and is being used in rheumatic conditions. When injected into joints pain and swelling are reduced for extended periods of time. Compound F is said not to have some of the undesirable side reactions that cortisone has.

Sex Hormones of the Adrenal Cortex. Besides producing the hormones already discussed, the adrenal cortex produces several hormones that have activities similar to the sex hormones. Both male and female sex hormones are produced in the adrenal cortex, although they are not identical with those produced in the ovaries and testes. A condition known as **adrenal virilism** develops in women when their adrenal cortex produces too much male sex hormone. In this condition women take on masculine characteristics. A similar condition may occur in men when the adrenal cortex produces too much female sex hormone. In this condition the man takes on female characteristics. Sometimes in tumor of the cortex, male sex hormone is overproduced in both the male and female. If this situation exists in the male, the male characteristics are accentuated; if in the female, the female characteristics are superseded by those of the male. If the adrenal cortex produces too much male sex hormone in young boys they reach sexual maturity early. A compound called **adrenosterone** has been isolated from beef adrenals which has male sex hormone activity.

The Reproductive Organs

It has been known for a long time that the testes and ovaries have important effects on the animal body. It is now known that these effects are due to hormones which these organs produce.

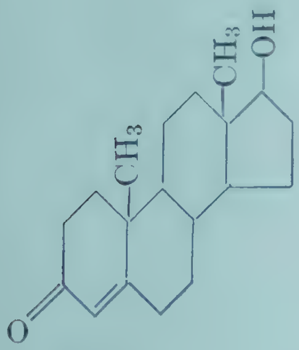
Testes; Effects of Removal. If the testes are removed from a young animal, the most noticeable result is that the secondary male sex characteristics do not develop. In man the beard fails to grow, the tone of the voice remains childlike, and the individual develops female characteristics. The breasts and hips become large, and the body form resembles that of the female. In cocks the wattles, comb, and spurs fail to develop normally, and in stags the antlers do not grow.

It is common practice in livestock raising to castrate young animals which are grown for meat production. Castration slows down the metabolic rate and quiets the temperament, so that the animal puts on weight more rapidly. The meat of such animals is also of better quality than that of normal animals. The capon of the poultry man and the steer of the beef raiser are examples of the effects of castration.

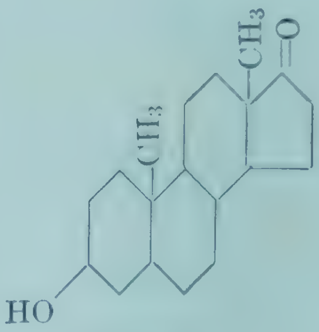
Male Sex Hormones. Extracts of testes and urine have been prepared which, when injected into capons, have a profound effect on secondary sex characteristics, especially the growth of the comb. From these extracts three compounds have been isolated which are believed to be the male sex hormones. These compounds are assayed by determining their potency in stimulating the growth of the comb of capons.

The hormone isolated from the testes, called **testosterone**, is much more potent than the other two. It is considered to be the primary male sex hormone. Those isolated from the urine are **androsterone** and **dehydroandrosterone**, androsterone being the more potent. These are thought to be derived from testosterone by oxidation.

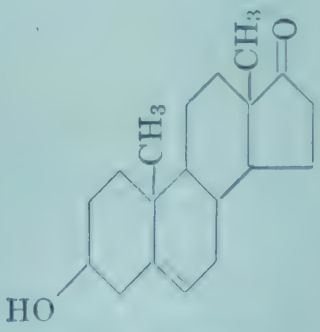
The chemical structure of these compounds has been determined, and they have been found to be cyclopentanoperhydrophenanthrene derivatives.



Testosterone



Androsterone



Dehydroandrosterone

Besides these three compounds, several compounds prepared from sterols are known that have male sex hormone activity. It will also be recalled that the adrenal cortex produces a male sex hormone called **adrenosterone.**

The testes are stimulated to produce testosterone by a hormone secreted by the anterior pituitary gland.

The Estrus Cycle. Before considering the female sex hormones, it is important to know what happens in the reproductive organs during the estrus cycle. The estrus cycle is the series of changes that take place in the reproductive organs of the female over a period, in human beings, of about 4 weeks. The estrus cycle first starts at puberty. Before puberty the ova are embedded in the ovary in the form of minute **Graafian follicles.** At puberty, as a result of stimulation by a hormone of the pituitary gland, some of these Graafian follicles enlarge and move toward the surface. The tissue of the Graafian follicles produces a female sex hormone called **estradiol.** Finally the follicle ruptures; the ovum is discharged and enters the mouth of the Fallopian tube leading to the uterus.

After rupturing, the follicle fills with blood, later becomes yellow, and for a time increases in size, forming a **corpus luteum.** The corpus luteum produces a second female sex hormone called **progesterone.** If the discharged ovum is not fertilized or, in other words, if pregnancy does not occur, the corpus luteum continues to grow for about a week and then regresses. This stage is followed by the growth and development of another ovum and Graafian follicle, and the process is repeated every 4 weeks in a normal woman.

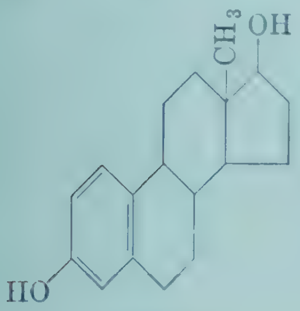
Accompanying the changes going on in the ovary, profound changes take place in the uterus, undoubtedly due to the hormones produced in the Graafian follicles and in the corpus luteum. The muscular walls and the epithelial lining of the uterus thicken, preparing it for the reception of the ovum. If fertilization of the ovum does not occur, about 2 weeks after ovulation the uterine epithelium disintegrates and is discharged. This discharge of the uterine epithelium, which is accompanied by bleeding, is called the **menses,** and normally lasts about 5 days. About 14 days after the onset of menses ovulation occurs again, and the cycle is repeated.

In some animals such as the rat the changes in the ovaries are accompanied by changes in the vagina due to the estrogenic hormones. Before the Graafian follicle starts to develop, a vaginal-smear shows many leucocytes, some mucus, but no characteristic flat cells without nuclei. When the Graafian follicle reaches its maximum size and produces its greatest quantity of hormone, a vaginal smear shows no leucocytes but much mucus and many flat nonnucleated cells. If estradiol is injected into rats from which the ovaries have been removed, the vaginal changes will take place. Thus the vaginal-smear method is valuable in assaying estrogenic hormones.

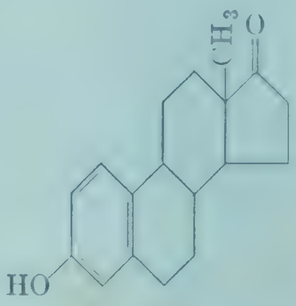
The hormones produced by the ovary also initiate development of the mammary glands.

If pregnancy occurs, the corpus luteum does not regress, but continues to grow. This condition prevents further ovulation, and the estrus cycle ceases.

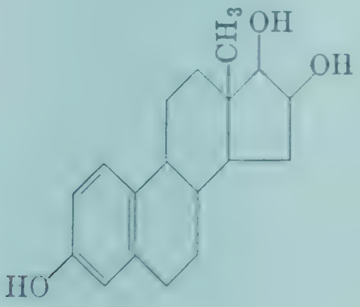
Follicular Hormones. When the ovaries are stimulated by a hormone from the anterior pituitary gland a Graafian follicle enlarges and produces hormones which initiate the estrus cycle. These hormones are usually referred to as estrogenic hormones. Five such hormones have been isolated from ovaries and urine.



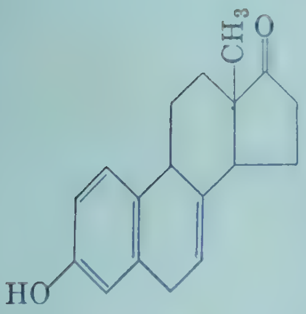
Estradiol



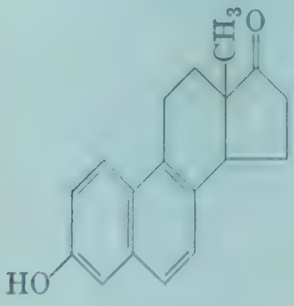
Estrone



Estriol



Equilin



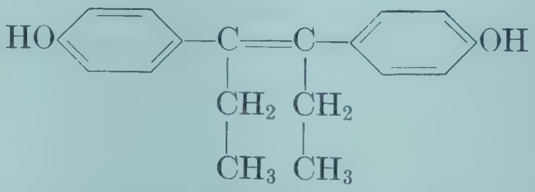
Equilenin

Estradiol is the most active of these hormones; it is six times as active as **estrone.** For this reason it is considered to be the original estrogenic hormone. **Estriol** is about one-hundredth as active as estrone and is found in the urine combined with glucuronic acid. It is considered the end product of estradiol metabolism. **Equilin** and **equilenin** are found in the urine of pregnant mares.

A comparison of the formula of estrone with those of the male sex hormones indicates a striking similarity. Estrone has been found in both female and male urine and blood. Pregnancy urine is especially rich in estrone. The urine of pregnant mares contains about ten times as much as that of pregnant women. The urine of stallions contains nearly twice as much estrone as that of pregnant mares.

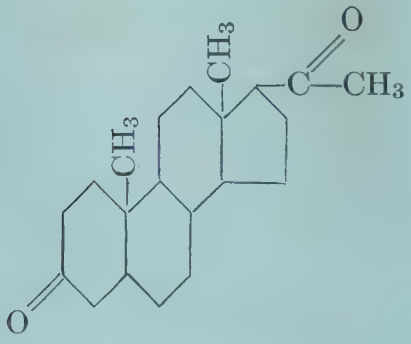
The first estrogenic hormone to be isolated in a pure form was prepared by Allen and Doisy in 1923. They called it **theelin** from a Greek word meaning female. It is now called estrone. They also isolated a closely related compound which they called **theelol.** Theelol is now called estriol.

Besides the naturally occurring estrogenic hormones described several synthetic compounds are known that have similar activities. One such compound which has found considerable use in medicine is **diethylstilbestrol.** It is easily synthesized and is cheaper than the naturally occurring hormones. It is about three times as active as estrone

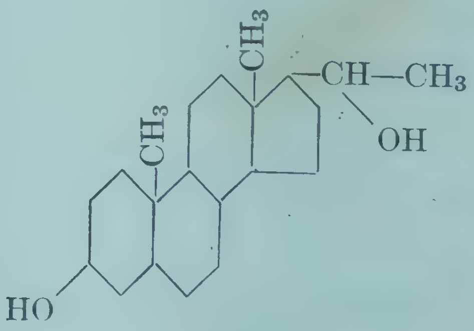


Diethylstilbestrol

Corpus Luteum Hormone. As mentioned earlier, after the Graafian follicle discharges its ovum a corpus luteum develops which produces another female sex hormone called **progesterone.** Its main function appears to be to continue the changes in the uterus started by the estrogenic hormones, preparing it for the implantation of the ovum. It also plays some part in the retention of the embryo in the uterus. If the



Progesterone



Pregnanediol

corpus luteum is destroyed during early pregnancy, abortion occurs. Progesterone stimulates the development of the mammary gland at puberty, and it is believed that this hormone plays an important part in the development of the mammary glands during pregnancy.

Progesterone is closely related chemically to pregnanediol, which has been isolated from pregnancy urine. It is very possible that pregnanediol is derived from progesterone by reduction.

The Placenta. When pregnancy occurs, the placenta, which is a new organ of internal secretion, develops. It is a round, flat organ which forms the attachment between the mother and the fetus and through which the mother nourishes the fetus. It produces a hormone, protein in nature, which is called the **anterior pituitarylike hormone** because it stimulates the growth of Graafian follicles, like the hormone of the anterior pituitary. This hormone is secreted in urine during pregnancy and is said to be the substance responsible for the **Ascheim-Zondek test** for pregnancy. In this test the suspected urine is injected into immature mice. In 4 days the mice are killed and the ovaries examined for corpora lutea, as indicated by bloody and yellowish spots, the presence of which indicates pregnancy. In this manner pregnancy may be determined within 2 or 3 weeks after conception has occurred. A modification of this test known as the **Friedman test** makes use of adult female rabbits.

The Pituitary Gland

The **pituitary** gland, often called the hypophysis, is a small organ about the size of a pea lying at the base of the brain. As it is connected to the brain, its removal is difficult without injury to the brain. Structurally it is composed of three parts: the **anterior lobe**, which is glandular in nature; the **posterior lobe**, which resembles nervous tissue; and the **pars intermedia**, which connects the two lobes. A discussion of the hormones of the pituitary is difficult because so many physiological functions have been attributed to it. Such a large number of the activities of the pituitary involve the control of other endocrine organs that the pituitary is spoken of as the **master gland** of the body. Since the two lobes and the pars intermedia of the pituitary have different physiological properties, they will be discussed separately.

ANTERIOR LOBE

Whether the pituitary is essential for life is doubtful. Early experiments in which the pituitary was removed resulted almost invariably in the death of the animal. In these cases death was probably due to injury to the brain. Later work, using more refined technique, indicates that animals may survive after the pituitary has been removed. If the anterior lobe of the pituitary is removed in a young animal, the most noticeable effect is that growth is checked; the animal remains infantile; the sexual organs do not develop. In dogs, puppy hair and milk teeth persist for long periods of time; the brain fails to develop normally, and there is low intelligence; carbohydrate, fat, and protein metabolism is altered; there is a slower basal metabolic rate; and body temperature is lowered. In human beings dwarfism may be associated with a subnormal functioning of the anterior lobe of the pituitary. Since the administration of anterior pituitary preparations will prevent the appearance of these symptoms, we may conclude that the anterior lobe of the pituitary produces a hormone which stimulates growth and metabolism. If the anterior lobe of the pituitary is removed from an adult, the effect is not so noticeable because the animal already has its growth. However, the metabolic changes occur.

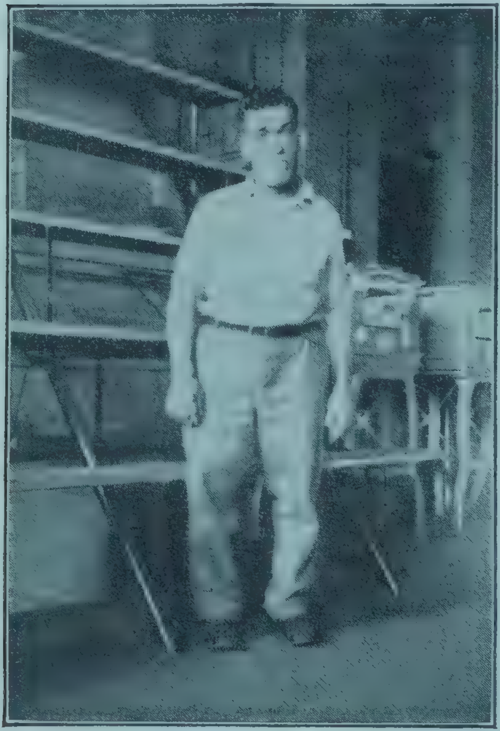


Fig. 38. Acromegaly. From *A Manual of Biochemistry* by J. F. McClendon. Courtesy of Dr. J. F. McClendon.

If the anterior lobe of the pituitary is too active, remarkable changes occur. In young people the long bones become longer than normal and a condition known as **gigantism** results. There is one case on record of a young man 19 years of age being 8 feet 4 inches tall and weighing 390 pounds. In adults the long bones do not grow, but the bones of the face, hands, and feet grow, producing a condition known as **acromegaly.** (See Fig. 38.) The term acromegaly comes from two Greek words meaning large extremity. In conditions such as those just described, partial removal of the anterior lobe by surgery has been found effective treatment.

All of the hormones of the anterior lobe of the pituitary appear to be protein in nature. Several have been prepared in pure form. Just how many hormones are present is not known. That there are several is indicated by the fact that various preparations have different activities. The following are the main hormones recognized as present in the anterior lobe of the pituitary:

Growth Hormone. If this is lacking in a young animal, dwarfism results. If it is present in too large quantities, gigantism or acromegaly results.

Thyroid-Stimulating Hormone. This hormone controls the thyroid gland. If it is lacking, the thyroid gland atrophies and the metabolic rate becomes low. If it is injected into animals, the thyroid gland enlarges and all the symptoms of toxic goiter may appear.

Parathyroid-Stimulating Hormone. Extracts of the anterior lobe of the pituitary are said to cause growth and increased activity of the parathyroid glands.

Pancreas-Stimulating Hormone. Extracts of anterior pituitary cause a growth of the islet tissue of the pancreas with an increased production of insulin, indicated by low blood-sugar values.

Diabetogenic Hormone. It has already been pointed out that several hormones are involved in the control of the blood-sugar level. Insulin tends to decrease the blood-sugar level; adrenaline tends to increase it. There is also a hormone of the anterior pituitary which tends to increase the blood-sugar level; it is called the diabetogenic hormone. It acts by depressing the activity of insulin that lowers the blood-sugar level.

Ketogenic Hormone. This is a hormone which increases the production of acetone bodies. It is said to lower the level of blood lipids, depress the metabolic rate, and increase the specific dynamic effect of proteins.

Adrenocorticotropic Hormone (ACTH). The anterior pituitary produces a hormone which stimulates growth of the adrenal cortex. It will be recalled that the adrenal cortex produces several hormones that are essential for health and even life. Addison's disease, rheumatoid arthritis, rheumatic fever, and various allergies are thought to be associated with a deficiency of adrenal cortex hormones. One method of treating these diseases is to use ACTH which stimulates the adrenal cortex to produce its hormones.

Lactogenic Hormone. This hormone has been prepared in crystalline form and is called **prolactin.** It is the hormone that causes the secretion of milk by the mammary glands. During pregnancy female sex hormones stimulate growth of the mammary glands and inhibit the production of prolactin by the anterior pituitary. At parturition, when the production of female sex hormones falls off, prolactin is secreted which stimulates milk production.

Gonad-Stimulating Hormones. The gonads are the sex glands, the ovaries, and the testes. In the discussion of the female sex hormones it was pointed out that the formation of Graafian follicles and of corpora lutea was controlled by hormones of the anterior pituitary. In like manner the male sex organs are stimulated by anterior pituitary extract. Two hormones have been prepared from the anterior pituitary that are responsible for this stimulation. They are both glycoproteins. They are the same in both male and female. One called the **follicle-stimulating hormone** (FSH) stimulates the production of Graafian follicles of the ovary in the female and growth of the sperm-forming tissue of the testes in the male. The other called the **luteinizing hormone** (LH) stimulates the formation of corpora lutea in the ovary and the production of testosterone, the male sex hormone, in the testes.

Young animals may be caused to mature sexually at an early age by the administration of anterior pituitary extract. If the anterior lobe of the pituitary is removed from a young animal, it remains infantile; if removed from an adult, the sex organs atrophy.

POSTERIOR LOBE

Extracts of the posterior lobe of the pituitary have three well-recognized physiological activities. First, they stimulate the contraction of the muscles of the uterus, bladder, and intestines, and all other smooth muscles. This property is known as **oxytocic activity;** in medicine these extracts are employed to bring on labor in childbirth. Second, they cause a rise in blood pressure. This property is known as **pressor activity;** it is utilized in medicine for overcoming surgical shock. Third, these extracts have an effect on the kidneys known as the **diuretic-antidiuretic activity.** If rabbits are fed on succulent foods, injections of small amounts of these extracts will cause a temporary flow of urine. This is an example of the diuretic activity. In diabetes insipidus, a disease characterized by an enormous flow of urine, injections of these extracts will reduce the flow. This is known as the antidiuretic activity.

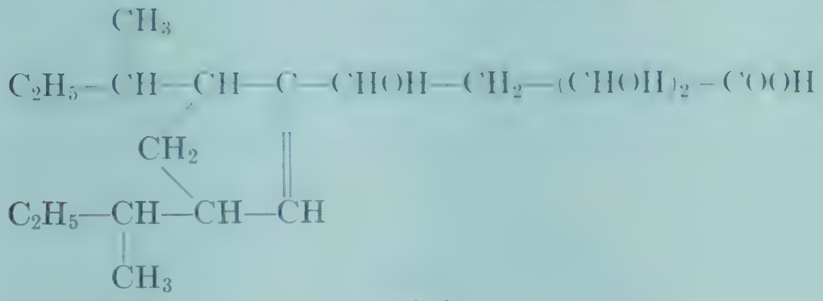
Whether all the activities of the extracts of the posterior lobe of the pituitary are due to a single hormone or to more than one is a question upon which authorities disagree. Abel believed that there is a single hormone. Kamm and his co-workers have been able to obtain two hormones which are quite distinct in their properties. They have been unable to designate their chemical structure but have found them to be basic in character and have called them **α- and β-hypophamines.** It is now believed that these hormones are protein in nature, specifically polypeptides with molecular weights of about 1000. α-Hypophamine is the oxytocic principle; β-hypophamine is the pressor principle and also is responsible for the diuretic-antidiuretic activity. α-Hypophamine is also known as **oxytocin** and **pitocin**; β-hypophamine as **vasopressin** and **pitressin.**

Pars Intermedia

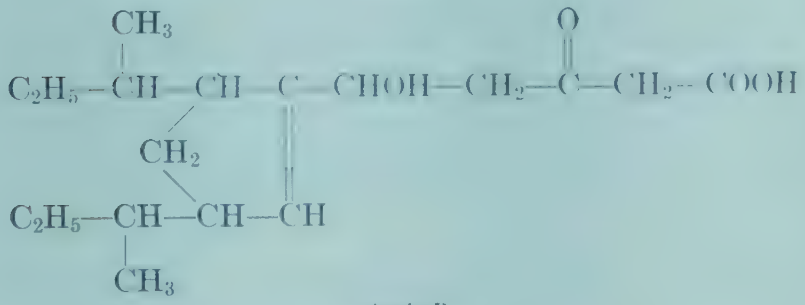
A hormone called **intermedin**, which has the interesting property of dilating pigment cells of the skin of frogs and the scales of fish, has been prepared from the pars intermedia. The injection of this hormone into a frog increases the size of the pigmented spots on the frog's back. Just what the function of this hormone is in higher animals is not known.

Plant Hormones

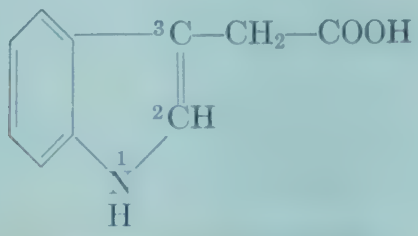
Although we usually think of hormones as being animal products it should be pointed out that plants also produce substances that have a definite influence on such processes as growth. They stimulate the



Auxin-A



Auxin-B



Heteroauxin (indole-3-acetic acid)

growth of leaves in grain sprouts and of roots in cuttings and prevent the premature fall of leaves in certain ornamental plants. These hormones are not produced in special organs, as in animals, but in rapidly growing tissues such as the tip of leaves, root tips, and buds.

Kögl, who has done important work in this field, named these growth-promoting substances **auxins** from a Greek word meaning "to increase." Three auxins found in plants are recognized. They are **auxin-A, auxin-B,** and **heteroauxin.**

Auxin-A is found in the tips of sprouting oats and corn and in fungi and yeast, auxin-B in malt and maize oil, and heteroauxin in wheat germ and molds. All three of the auxins have been found in human urine.

In 1939 Bonner isolated a compound from green string beans which stimulated cell growth in injured plant tissues. He called it **traumatic acid,** and it is often referred to as a "wound hormone." It has the following formula:

$$HOOC-CH=CH-(CH_2)_8-COOH$$

Traumatic acid

Besides the naturally occurring plant hormones many chemicals are known that stimulate growth in plants. Among these may be mentioned **ethylene,** which hastens the ripening of fruits and stimulates root development, and **indole-3-butyric acid** and **α-naphthalene acetic acid** which stimulate root growth. Naphthalene derivatives are used to prevent the premature fall of apples and pears.

It is interesting to note that certain vitamins stimulate plant growth. Important among these are **thiamine, niacin,** and **pyridoxine.** They stimulate root development in certain plants.

From what has been said it is obvious that plant hormones should have considerable application in various branches of agriculture such as horticulture and pomology. The application of traces of plant hormones to the soil improves root development in young plants and as a consequence increases the vigor of the entire plant.

REVIEW QUESTIONS

1. What is an endocrine organ? Name the important endocrine organs and tell where each is located.
2. What is a chalone?
3. Name five hormones of the digestive tract and indicate what each does.
4. Discuss the structure of the pancreas.
5. What happens when the pancreas is removed?
6. What is the chemical nature of insulin?
7. Define a unit of insulin.

8. What is meant by hyperinsulinism?

9. Name three hormones other than insulin that influence the blood-sugar level.

10. What is alloxan diabetes?

11. Who first isolated thyroxine, and who first determined its chemical structure?

12. What is thyroglobulin?

13. What are the symptoms of hypo- and of hyperthyroidism?

14. What is the difference between simple and exophthalmic goiter?

15. How may exophthalmic goiter be treated surgically and chemically?

16. Discuss the relation of iodine to goiter.

17. Discuss the parathyroid glands as endocrine organs.

18. What is von Recklinghausen's disease?

19. Name the two parts of the adrenal glands.

20. What is meant by chromaffine tissue?

21. What is the function of adrenaline?

22. What is ephedrine?

23 What is Addison's disease?

24. What is the relation of Addison's disease to sodium metabolism?

25. Name several physiological effects associated with hypofunction of the adrenal cortex.

26. Name six important hormones found in the adrenal cortex.

27. What is cortisone and for what is it used?

28. What is adrenal virilism?

29. What is the effect of the removal of the testes?

30. What is the original male sex hormone? Name two other male sex hormones.

31. How are the male sex hormones assayed?

32. Describe the estrus cycle.

33. Describe the functions of estradiol and progesterone.

34. Name four hormones isolated from urine that are thought to be derived from estradiol.

35. Name a synthetic estrogen commonly used in medicine.

36. What is the origin of pregnanediol?

37. Discuss the placenta as an endocrine organ.

38. Describe the Aschheim-Zondek and the Friedman tests for pregnancy.

39. Name the three parts of the pituitary gland.

40. What hormones are found in the anterior lobe of the pituitary gland, and what is the function of each?

41. What is acromegaly?

42. Name two hormones of the posterior lobe of the pituitary gland. What is the function of each?

43. What is diabetes insipidus?

44. What is the function of intermedin?

45. Name four naturally occurring plant hormones. What is their main function?

46. Name several synthetic compounds that have been used to stimulate plant growth and state some of the uses to which they have been put.

47. Name three vitamins that stimulate plant growth. What part of the plant do they stimulate?

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21

Vitamins

At the beginning of the twentieth century much of the fundamental work on the calorific requirements of the animal body was completed. It was generally believed that an adequate diet was one which furnished the necessary calories, together with protein and mineral salts in sufficient quantities to supply the body needs. Emil Fischer, through his monumental work on proteins, complicated the picture somewhat by pointing out that proteins varied in their amino acid content. This led many workers to investigate the nutritional value of the various amino acids. As a result of this work we now know that the various proteins differ widely in their nutritive value and that it is not sufficient to include a given weight of any protein in the diet. Protein quality is just as important as protein quantity.

During the present century the main advances in our knowledge of nutrition have been in the field of **vitamins.** It has been shown that an adequate diet for health and proper growth must contain minute quantities of organic substances other than carbohydrates, fats, proteins, and salts.

Because the first of these substances studied was found to be essential for life and because it was an amine it was given the name of vitamine. As work progressed other substances were found that must be present in the diet for normal health and growth, and they were all referred to as vitamines. Since most of these are not amines several workers in the field objected to the term vitamine as a name for this group of food factors. However, the use of the word had become so widespread that it was retained with the elimination of the final *e*. Modern literature speaks of these essential food factors as vitamins.

In many respects vitamins are like hormones; both are necessary for normal metabolism. They differ in that vitamins must be supplied in the diet whereas hormones are produced in special organs of the body. Several of the vitamins have been shown to be the functional group of enzymes involved in biological oxidations. It is possible that eventually all hormones and vitamins will turn out to be associated with enzyme systems.

Early History. Although the scientific study of vitamins is a recent development, it is now known that their value has been appreciated for several hundred years. In 1720 the use of citrus fruits as a cure for **scurvy** was recommended by Kramer, an Austrian military physician. Even in those early days sailors knew that, if they were without fresh food for any considerable length of time, many of their number would be afflicted with ship **beriberi** and **scurvy.** In 1882 an experiment was conducted by the Japanese navy to study the effect of diet on the occurrence of ship beriberi. During a world cruise there were many cases of beriberi on board the ship furnishing the old regulation diet, but on ships in which fruits, vegetables, barley, and meat had been included in the diet very little beriberi occurred.

The scientific development of the study of vitamins dates back to 1881, when Lunin demonstrated that mice could not live on a diet composed of purified carbohydrates, fats, proteins, and salts. On the addition of milk to the diet the mice developed normally. Lunin concluded that milk contained some unidentified substance essential to normal life. In 1906 Hopkins of England conducted experiments similar to those of Lunin and concluded, as did Lunin, that milk contained some substance necessary for life other than those substances then recognized as essential.

Perhaps the most important early work on vitamins was that of Eijkman, a Dutch physician working in the Dutch East Indies in 1897. One of the most serious diseases with which he had to contend was beriberi. He showed that chickens fed on polished rice developed typical beriberi symptoms. If the chickens were fed on unpolished rice, they did not contract the disease. He also showed that the disease could be cured or prevented by the administration of an alcoholic extract of rice bran. He believed that the central portion of the rice kernel contained a poison which was causing the disease. The bran coat of the rice, which was removed during the polishing process, he believed to contain a substance capable of neutralizing this poison. We now know that rice bran contains a vitamin which is not present in the other parts of the kernel.

The name vitamine was suggested by Funk, a Polish chemist, who repeated Eijkman's work in 1911 and isolated from rice polishings a crystalline substance, aminelike in chemical properties, which had the power of curing avian beriberi. Later work has shown that Funk's crystals were not the pure vitamin.

Recent Developments. In 1913 Osborne and Mendel and McCollum and Davis discovered that certain animal fats, such as butterfat and egg oil, contained a growth-promoting factor not possessed by the vegetable oils. By 1916 McCollum and Kennedy had suggested that there were two unidentified food factors. The growth-promoting factor found in butterfat they designated as **fat-soluble A,** and the water-soluble factor which cures beriberi and is found in rice bran and yeast they called **water-soluble B.** At the present time these two factors are known as **vitamin A** and **vitamin B_1 or thiamine.**

Although it has been known for many years that scurvy is in some way related to the diet, it was not until about 1912 that it was recognized as a vitamin-deficiency disease. Holst and Frölich, working in Norway (1907 to 1912), showed that guinea pigs fed on cereals and hay developed scurvy. The disease could be prevented and cured by feeding fresh foods. Other workers verified this observation, and the scurvy-preventing factor was called **vitamin C.**

The next disease investigated that was found to be due to a vitamin deficiency was **rickets.** This disease, like scurvy, had been known for a long time. It occurs especially in young children and is characterized by poor calcification of the bones, which often results in bowlegs. The antirachitic vitamin, in accordance with the system already in use for naming vitamins, was called **vitamin D.**

In 1922 Evans and Bishop discovered a new vitamin which is necessary in the diet for reproduction. Without it animals become sterile. This vitamin was called **vitamin E** or the antisterility vitamin.

Since 1922 many new vitamins have been discovered. What was originally thought to be a single vitamin, namely, vitamin B, has been found to be a complex made up of several vitamins, and this group of vitamins is often referred to as the **vitamin B complex.**

Perhaps the most important recent experiments in the field have been those leading to the isolation of most of the known vitamins in the pure form and the determination of their chemical structure. Several of the vitamins are now being synthesized in the chemical laboratory and are replacing those obtained from natural sources. Also much work is being done to determine how vitamins function in metabolic processes. Many of the vitamins have been shown to be a part of enzyme systems.

Because the vitamins are becoming so numerous, and also because the chemical constitution of most of them is known, it is becoming common practice to refer to them by their chemical names. In fact, for some of the recently discovered vitamins there is no alphabetical designation.

The vitamins may be classified into two main groups on the basis of solubility. The fat-soluble vitamins include vitamins A, D, E, and K; and the water-soluble vitamins include the vitamins of the B complex, C, and P.

Fat-Soluble Vitamins

VITAMIN A

Effects of Deficiency. The vitamins are dietary factors which, if lacking, manifest their absence by certain diseased conditions or abnormal body functions. If vitamin A is lacking in the diet, one of the first effects is a change in the epithelial cells of the mucous membranes of the body. These membranes lose their power of secreting moisture and become dry and hardened. This drying up of the mucous membranes is usually first noticed in the eyes and eyelids, which become inflamed and filled with pus. (See Fig. 39.) Finally blindness results. Because of this characteristic drying up of the membranes of the eyes the condition has been called **xerophthalmia,** which means dry eyes. Infections with pus formation also occur in the glands at the base of the tongue, in the sinuses, and in the ears. Other sites of infection are the lungs and respiratory tract, the alimentary canal, and the urinary tract. A generous supply of vitamin A in the diet aids in preventing colds and other respiratory infections. Lack of vitamin A favors the formation of urinary calculi. In general it may be said that one of the main functions of vitamin A is to promote the development of

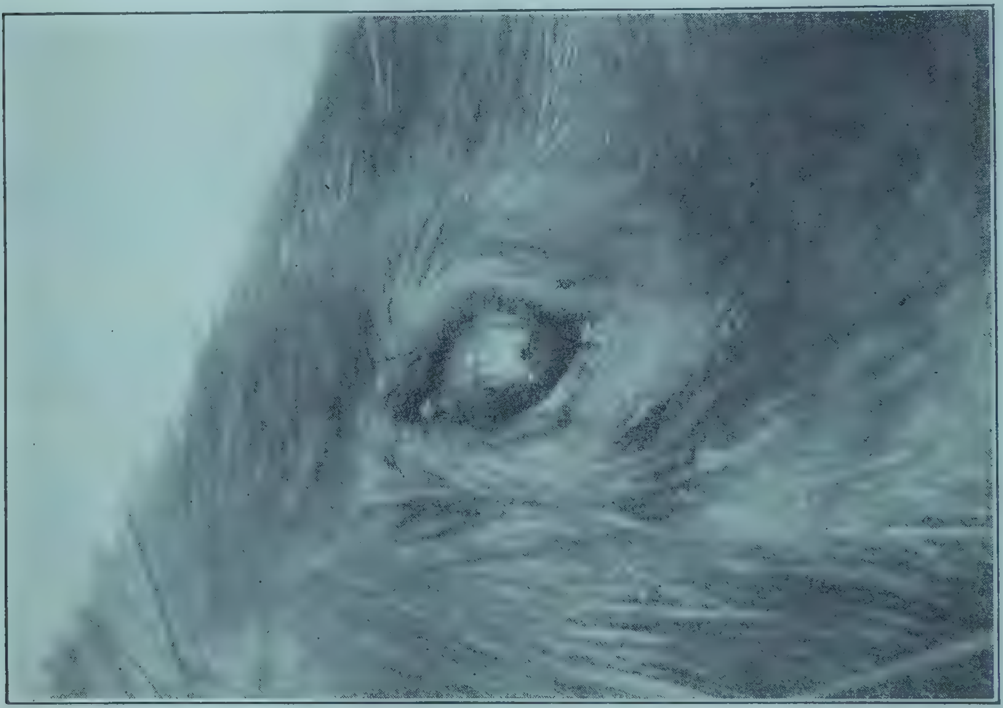


FIG. 39. Rat's eye showing xerophthalmia. Courtesy of Dr. N. B. Guerrant.

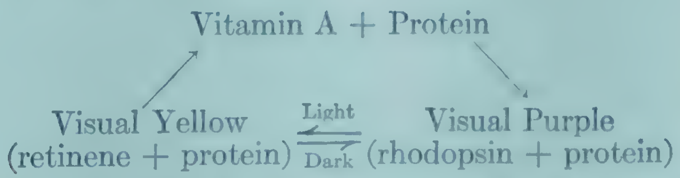
healthy mucous membranes which are not easily invaded by pathogenic organisms.

Because vitamin A appears to aid the body in avoiding certain infections, it has been called the **anti-infective vitamin.** Some persons object to the term because it implies that vitamin A has bactericidal properties. This, of course, is not the situation; vitamin A prevents infection by aiding in the maintenance of normal, healthy mucous membranes.

Other disturbances from a lack of vitamin A are physical weakness, loss of appetite, failure of digestion, diarrhea, and retardation of growth and development. Vitamin A is necessary for normal reproduction and lactation. In its absence sterility results because of infections and changes in the mucous membranes lining the genital tract.

Deficiency of vitamin A in the diet has been shown to be associated with a condition of the eyes known as **night blindness.** It is common knowledge that in driving a car at night one is often temporarily blinded by approaching headlights. It has been found that the time required to recover normal vision under these conditions is longer for individuals who are deficient in vitamin A than for those who are well supplied with this vitamin.

This phenomenon has been explained as follows: In the retina of the eye there is a pigment called **visual purple,** which is essential for normal vision. Visual purple is a chromoprotein composed of a rose-colored pigment, **rhodopsin,** and a protein. Rhodopsin contains vitamin A or requires vitamin A for its synthesis. When visual purple is exposed to strong light, it is changed to **visual yellow,** which is a complex composed of a pigment, **retinene,** and a protein. In order for normal vision to return, visual purple must be regenerated. In the dark a part of the visual yellow is reconverted into visual purple, but some is converted into vitamin A and other products. The vitamin A formed is used to synthesize visual purple, but in the cycle some vitamin A is lost and more must be supplied in the diet. If a generous supply of vitamin A is present in the retina, the normal amount of visual purple is quickly regenerated. This discussion may be represented diagrammatically as follows:



Sources. An excellent source of vitamin A is halibut-liver oil, sold on the market as haliver oil. Cod-liver oil is also rich in this vitamin.

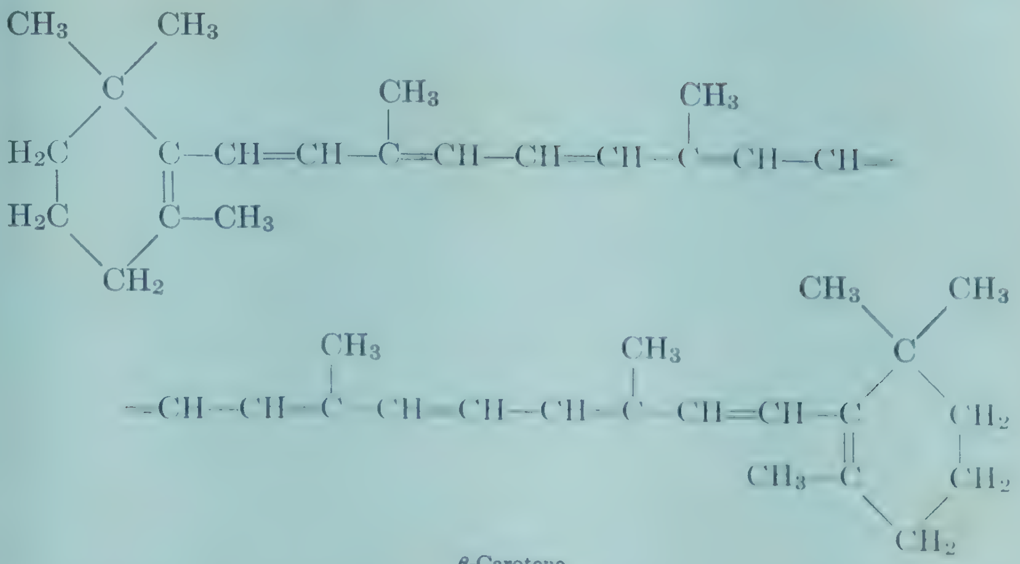
The liver oils of many other species of fish are rich in vitamin A; in fact, some fish-liver oils are several hundred times as potent in vitamin A activity as cod-liver oil. The vitamin A in fish-liver oils is said to originate in certain unicellular marine organisms which are able to synthesize this vitamin. These organisms serve as food for marine plankton which are consumed by small fish; these in turn serve as food for larger fish.

Vitamin A or its precursors are widely distributed in our natural foods. Free vitamin A does not occur in plants, but in its place are compounds that are converted into vitamin A in the animal body. These precursors of vitamin A are often called **provitamins A.** In animal foods such as milk, butter, and eggs both the vitamin and its precursors are present. In fish-liver oils the vitamin itself is present rather than its precursors. In speaking of the vitamin A content of foods, it is customary not to distinguish between the free vitamin and its precursors. The precursors of vitamin A are discussed on the following pages.

In the vegetable foods vitamin A is frequently present in direct proportion to pigmentation. Yellow corn contains more vitamin A than white corn. The green outer leaves in a head of lettuce have thirty times as much vitamin A as the crisp inner portion. The vegetable escarole has the highest vitamin A content of any vegetable. Spinach and kale are also excellent sources. Of the root crops, carrots and yellow sweet potatoes are rich in this vitamin. Green peas, string beans, and Brussels sprouts contain appreciable quantities of vitamin A. Among the fruits bananas, cantaloupes, watermelons, tomatoes, and cherries contain about as much vitamin A as green peas and string beans. Most of the cereals and nuts are very low in vitamin A. Animal fats are as a rule richer in vitamin A than vegetable oils. Vegetable oils which have been hydrogenated are especially poor in this vitamin. With the exception of liver, meats are a poor source of vitamin A. Egg yolk and butterfat are rich in this vitamin. Dairy products are the most important source of vitamin A in our diet.

At the present time vitamin A_1 is being synthesized; and the synthetic product is becoming an important source of this vitamin.

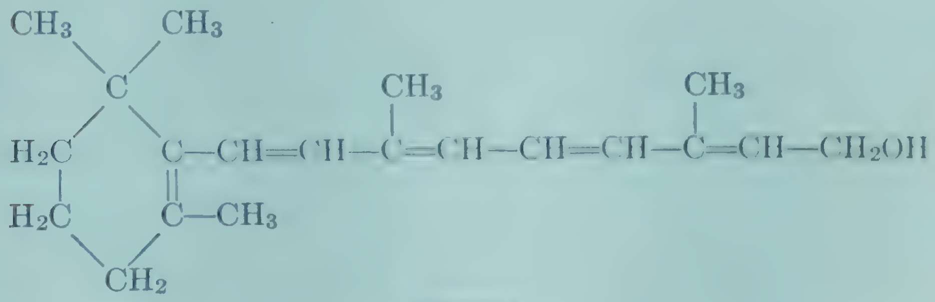
Chemical Constitution. Vitamin A, one of the fat-soluble vitamins, is related chemically to the hydrocarbon **β-carotene**, which is the characteristic pigment in carrots. It is interesting to note that the occurrence of vitamin A is closely associated with pigmentation in plants. β-Carotene has the empirical formula $C_{40}H_{56}$, and its constitution is shown by the following formula:



β-Carotene

β-Carotene is not vitamin A but, when eaten by an animal, is converted into this vitamin in the tissues, especially those of the liver and intestine. Both β-carotene and vitamin A may be stored in the body, particularly in the liver.

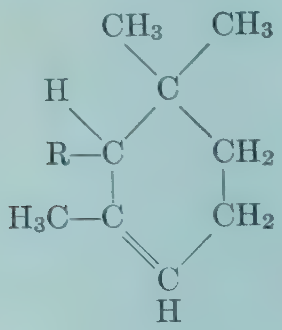
If a molecule of β-carotene were split in the center and the end carbons of the resulting compounds were oxidized to alcohols, two molecules of vitamin A should result. Thus 1 gram of β-carotene should be converted into about 1 gram of vitamin A. In other words, β-carotene should have the same biological value as vitamin A. Experiments have shown, however, that β-carotene has about one-half the potency of vitamin A. This indicates that only about one-half of the β-carotene fed is converted into vitamin A in the body.



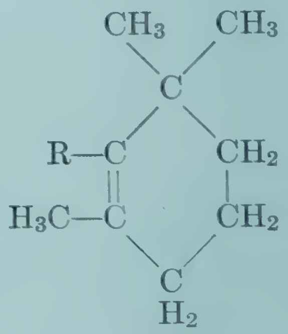
Vitamin A

Carotene exists in three forms, known as α-, β-, and γ-carotene. All are capable of producing vitamin A in the body. β-Carotene is a symmetrical compound made up of a long, unsaturated carbon chain with identical ring structures, known as β-ionone rings, at each end of the carbon chain. α- and γ-Carotene differ from β-carotene in that one of the ring structures has been altered. In α-carotene the position of the double bond in one of the rings has been changed. This ring is known as an α-ionone ring. In γ-carotene one of the rings is open. A fourth precursor of vitamin A is cryptoxanthine, a pigment closely related chemically to the carotenes. It differs from β-carotene in that one of the rings is a β-ionone ring with an hydroxyl group attached to it.

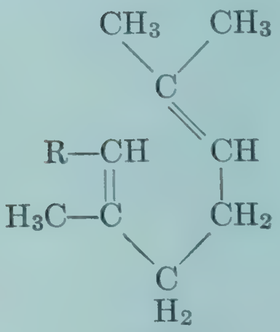
Since all these precursors of vitamin A differ only in the structure of one of the rings, their relationships may be shown by giving the structures of these rings.



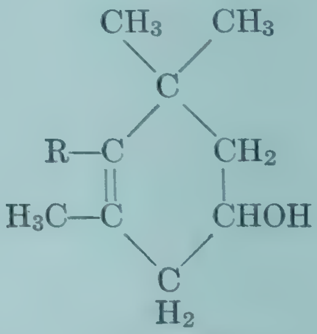
α-Carotene
(α-ionone ring)



β-Carotene
(β-ionone ring)



γ-Carotene
(open ring)



Cryptoxanthine
(β-ionone ring
with hydroxyl)

Apparently the ring structure given for β-carotene is essential for vitamin A activity. Since β-carotene contains two such rings, it should be capable of forming two molecules of vitamin A. Since α- and γ-carotene and cryptoxanthine have only one of these rings, they should be capable of forming only one molecule of vitamin A each. As stated above β-carotene has about one-half the potency of pure vitamin A α-Carotene and cryptoxanthine have about one-fourth the potency of the pure vitamin. Thus all the carotenes lose about one-half of the vitamin A that theoretically they should produce when converted into vitamin A in the body.

Vitamin A was prepared in a crystalline form in 1936 by Holmes and Corbet. The crystals are light yellow in color.

Wald, working on the pigments in fish eyes, found a new kind of vitamin A present in the eyes of fresh-water fish. He suggested that the common variety, found in salt-water fish, be called **vitamin A_1** and the new variety **A_2**. He has shown that the pigments present in the retinas of marine and fresh-water fish differ. The former contain **rhodopsin** and the latter contain a different pigment which he has called **porphyropsin.** When rhodopsin breaks down it gives vitamin A_1, whereas porphyropsin gives vitamin A_2. The difference in chemical structure between vitamins A_1 and A_2 is still in doubt.

Assay. In order to compare foods on the basis of their vitamin content, it is essential that some unit of measurement be adopted. In the past much confusion has resulted from the use of several units of measurement. In this book we shall speak of vitamin activity in terms of International units (I.U.) wherever these are available. The potency of a food in a vitamin which is available in the form of a pure chemical compound is expressed in terms of milligrams or micrograms of the pure compound present in a unit quantity of the food. A microgram is 0.001 of a milligram and is sometimes referred to as a γ (gamma). An International unit of vitamin A is equivalent to 0.344 γ of pure vitamin A_1 acetate. This is equivalent to 0.30 γ of pure vitamin A_1. The International standard used in assaying foods for their vitamin A activity is a solution of vitamin A_1 acetate in cottonseed oil. For determining provitamins A, the International standard is β-carotene, and an International unit is equivalent to 0.6 γ of pure crystalline β-carotene.

Of the various methods for assaying foods for their vitamin A content the biological method is perhaps the most reliable. In this method young rats are placed on a diet complete in every respect except that it contains no vitamin A. When the body supply of vitamin A is depleted, the rats cease to increase in weight, and they develop the eye disease known as xerophthalmia. At this point some of the rats are fed in addition to the basal diet a known number of units of the International reference standard of vitamin A. These rats start to gain in weight and the eye disease is cured. At the same time other vitamin A starved rats are fed in addition to the basal diet various fixed amounts of the food to be tested. The amount of food required to produce the same biological response as is obtained for the animals fed the International reference standard of vitamin A contains the same number of units of vitamin A as are contained in the standard fed.

Besides the biological method of vitamin A assay there are two other methods. One takes advantage of the fact that solutions of vitamin A exhibit a characteristic spectrographic absorption. The material to be tested is extracted with a suitable solvent, and the intensity of the absorption is determined. This intensity is proportional to the amount of vitamin A present. The second method, which is chemical in nature, depends upon the blue color produced when vitamin A is treated with antimony trichloride. This method is not considered accurate, but it is useful in obtaining a rough estimate of the vitamin A content of biological materials.

Requirements. It cannot be stated accurately what the vitamin A requirement is for a normal individual. Still less is known about the requirement in disease.

According to the Committee on Food and Nutrition of the National Research Council, the daily allowance of vitamin A for a normal adult should be 5000 I.U. This amount is equivalent to 3 mg. of β-carotene or 1.5 mg. of vitamin A_1. During the latter half of pregnancy this quantity should be increased to 6000 and during lactation to 8000 I.U. For children up to 12 years of age the allowance varies from 1500 to 4500 I.U. depending upon the age. (See Table 12.) The best way to insure an adequate intake of vitamin A is to include liberal quantities of pigmented vegetables and butter in the diet. If these foods are not available, the diet may be supplemented with halibut- or cod-liver oil.

VITAMIN D

Effects of Deficiency. Vitamin D is a dietary factor the absence of which is associated with an abnormal condition of the bones known as **rickets.** It has therefore been called the **antirachitic vitamin.** Rickets is a disease, common in infants, in which bones fail to calcify properly. The main mineral constituent of bones is calcium phosphate; therefore vitamin D is closely associated with calcium and phosphorus metabolism. When the bones fail to calcify correctly, they are soft and elastic and bend easily. In rickets the weight of the body causes the legs to bow. There is usually an enlargement of the joints, which may cause knock-knee. The ribs become beaded and may be deformed, producing a condition known as pigeon breast. The malformation of the pelvic bones in a female child may account for difficulty in childbirth in adulthood. When the disease develops in later life, it is known as **osteomalacia.**

Types of Rickets. Rickets is accompanied by definite changes in the composition of the blood. Uusally the phosphorus content of the blood serum is much lower than normal. Rickets in a person in whom the blood phosphorus is low and the calcium normal is known as **low-phosphorus rickets.** If the blood phosphorus remains normal but the calcium is low, the condition is known as **low-calcium rickets.** There is also a third type of rickets, in which both the phosphorus and calcium are low. Thus rickets is associated with low phosphorus or calcium values or both in the blood. It is, then, a disease characterized by improper calcium and phosphorus metabolism.

TABLE 12

RECOMMENDED DIETARY ALLOWANCES FOR SOME COMMON VITAMINS
(Revised, 1948)*

	Vitamin A, I.U.	Vitamin D, I.U.	Thiamine, mg.	Riboflavin, mg.	Niacin (Nicotinic Acid), mg.	Ascorbic Acid, mg.
Men (154 lb.)						
Sedentary	5000	...	1.2	1.8	12	75
Physically active	5000	...	1.5	1.8	15	75
With heavy work	5000	...	1.8	1.8	18	75
Women (123 lb.)						
Sedentary	5000	...	1.0	1.5	10	70
Moderately active	5000	...	1.2	1.5	12	70
Very active	5000	...	1.5	1.5	15	70
Pregnancy (latter half)	6000	400	1.5	2.5	15	100
Lactation	8000	400	1.5	3.0	15	150
Children up to 12 years						
Under 1 year	1500	400	0.4	0.6	4	30
1–3 years (27 lb.)	2000	400	0.6	0.9	6	35
4–6 years (42 lb.)	2500	400	0.8	1.2	8	50
7–9 years (58 lb.)	3500	400	1.0	1.5	10	60
10–12 years (78 lb.)	4500	400	1.2	1.8	12	75
Children over 12 years						
Girls, 13–15 years (108 lb.)	5000	400	1.3	2.0	13	80
16–20 years (122 lb.)	5000	400	1.2	1.8	12	80
Boys, 13–15 years (108 lb.)	5000	400	1.5	2.0	15	90
16–20 years (141 lb.)	6000	400	1.7	2.5	17	100

* Reprint and Circular Series, Number 129, October, 1948. Food and Nutrition Board, National Research Council, 2101 Constitution Avenue, Washington 25, D.C.

Factors Associated with the Etiology of Rickets. Vitamin D is not the only factor that is associated with rickets. The amounts of calcium and phosphorus in the diet, and especially the ratio of one to the other, are important. The best ratio of calcium to phosphorus in the diet to prevent rickets is 10 : 6. If this ratio is varied appreciably rickets will develop in an experimental animal receiving a deficiency of vitamin D much more readily than in one for which the ratio of these elements is proper. Sunlight has also been shown to take the place of vitamin D. Therefore vitamin D, the proper balance of calcium and phosphorus in the diet, and light must all be considered in studying rickets.

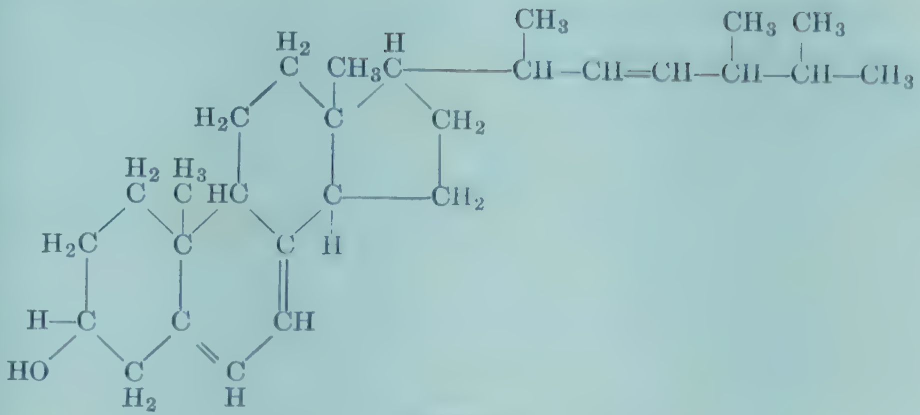
Light and Rickets. It has long been known that rickets is more prevalent in the winter than in the summer. It is also less prevalent at the equator than regions nearer the poles. Children raised in dark tenements and smoky cities are more subject to the disease than those raised in suburban homes. It is thus evident that sunlight has something to do with the prevention of rickets.

Investigation has shown that only the **ultraviolet** part of the sun's spectrum is active in preventing rickets. In diffuse daylight and in a smoky or cloudy atmosphere very little of the ultraviolet rays of the sun is present.

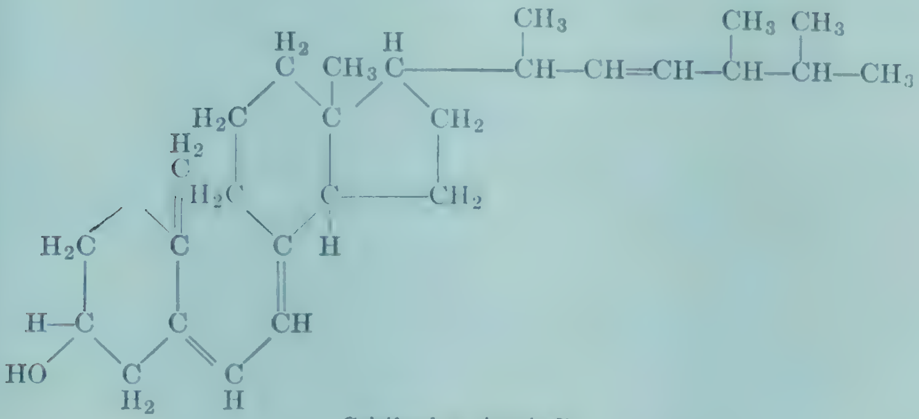
Ordinary window glass is not penetrated by the ultraviolet rays of the sun. Quartz or special window glass, which will allow the ultraviolet rays to pass through, must be substituted for ordinary glass if benefit is to be obtained from sunlight indoors.

Chemical Nature. It has been known for some time that the vitamin D potency of fats and oils is present in the unsaponifiable fraction of these lipids. Since cholesterol is one of the main constituents of the unsaponifiable fraction of animal fats, it was thought at first to be the parent substance of vitamin D. Irradiation of cholesterol with ultraviolet light gave it antirachitic properties. Later it was thought that it was not the cholesterol that was being converted into the vitamin by irradiation, but traces of another sterol that were present. This other sterol has been shown to be **ergosterol,** first found in the fungus ergot, which causes a disease of rye. Ergosterol is now known to be present in many other fungi and in yeast; in fact, it is widely distributed in plant and animal tisues. Ergosterol itself has no antirachitic potency; but, when irradiated with ultraviolet light, it becomes antiarchitic. It is now believed that ergosterol on irradiation is converted into vitamin D.

Askew and also Windaus isolated from irradiated ergosterol a substance which they considered to be the active principle of vitamin D. Askew called this substance **calciferol;** but Windaus, believing that several antirachitic substances existed, called it vitamin D_1. Later it was shown that this substance was not a pure compound but contained other sterols as impurities. Finally pure calciferol was isolated and was called by Windaus vitamin D_2. The formulas for ergosterol and calciferol show their relationship.



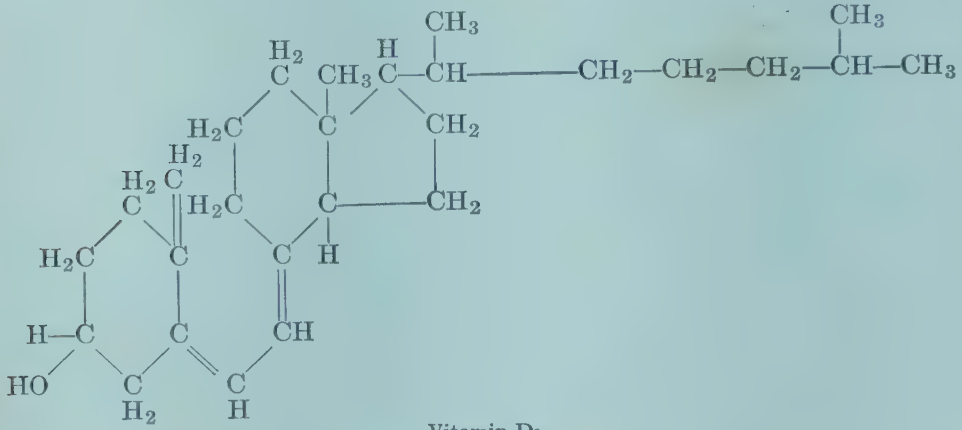
Ergosterol



Calciferol or vitamin D_2

Later work has indicated that calciferol is not the only compound that has antirachitic properties. Bills has shown that cod-liver oil and irradiated ergosterol are both efficient in curing rickets in rats, but in curing leg-weakness in chicks cod-liver oil is relatively much more potent than irradiated ergosterol. In other words, irradiated ergosterol is not so potent for chicks as for rats.

This work suggested that cod-liver oil contains an antirachitic substance different from calciferol. That this is the situation was shown by Waddell, who irradiated cholesterol and found that the product formed had antirachitic properties which were just as effective for chicks as for rats. It has now been shown that the activatable substance in cholesterol preparations is **7-dehydrocholesterol,** which differs from ergosterol in that it has no double bond and one less methyl group in the side chain. Windaus has prepared the active compound by irradiating 7-dehydrocholesterol and has called it vitamin D_3. Vitamin D_3 has been isolated from tunafish-liver oil; therefore apparently vitamin D_3 is the naturally occurring product. It should be pointed out that calciferol has not been isolated from natural products. It is quite probable, however, that it will be found to occur naturally. Vitamin D_3 is now made commercially and is used in the manufacture of vitamin preparations and in livestock feeds.



Vitamin D_3

Before leaving this subject it should be pointed out that other sterols have been prepared which may be made antirachitic by irradiation. Up to the present time about twenty different antirachitic compounds have been described. It is thus apparent that the antirachitic property is not due to a single compound but possibly to some characteristic group in many different molecules. This characteristic group may be the open-ring structure of calciferol and vitamin D_3.

Ergosterol, 7-dehydrocholesterol, and other sterols are sometimes spoken of as **provitamins D.** It is thought that some of these provitamins are present in small quantities in the animal body. When the body is exposed to ultraviolet irradiation, some of them are changed to vitamin D. Thus vitamin D is synthesized in the body, and there is no need for adding concentrates in the food provided that the body has been exposed to sufficient sunlight or artificial ultraviolet irradiation.

Viosterol. A very important commercial source of vitamin D, called **viosterol,** is now on the market. It is made by irradiating ergosterol and diluting it in maize oil. Viosterol is usually marketed in a strength expressed by the term 250 D, which means that it has a vitamin D potency two hundred and fifty times that of cod-liver oil. When viosterol is given in infant feeding, it should be noted that it is not a complete substitute for cod-liver oil, which is also a rich source of vitamin A.

Irradiation of Foods. Vitamin D may also be synthesized in foods by submitting them to ultraviolet irradiation. Many foods now on the market have had their vitamin D content increased by irradiation. Methods have been developed for irradiating liquid milk, which increases its vitamin D content many times. For the artificial production of ultraviolet light carbon-arc and mercury-arc lamps are used.

Sources. The most important natural source of vitamin D is cod-liver oil. It should be noted, however, that the cod is not the only fish whose liver oil is rich in this vitamin; in fact, the liver oils of many fish are rich sources. The body oils of sardine and herring have also been shown to have a high vitamin D potency. Egg yolk is another rich source of vitamin D, ranking second in importance to cod-liver oil. Milk and milk products, although relatively low in vitamin D, are important sources of this vitamin because of the large quantities consumed. Vegetable oils and plant foods are poor sources. The vitamin D content of milk is often increased by adding vitamins D_2 or D_3 directly to the milk.

Effects of High Intake. With the growing use of irradiation to increase the vitamin D potency of foods the question arises whether an overconsumption of this vitamin is injurious. There is evidence to indicate that excessive doses are definitely toxic and will cause calcification of other tissues besides bones. For example, the walls of the blood vessels may become calcified, producing a condition known as arteriosclerosis or hardening of the arteries. However, the amount of vitamin D required to bring about these effects is so large that there is little danger of injury from taking ordinary vitamin D preparations.

Stability. Vitamin D is relatively stable. It is not destroyed to any appreciable extent by heat or oxidation, as ascorbic acid is. Foods which have been cooked, dried, or stored retain most of their vitamin D potency.

Assay. In assaying foods for their vitamin D activity the biological method is followed. Since rats and chicks react differently toward different antirachitic substances, the choice of animal depends upon the information desired. When rats are used, young rats are placed on a diet lacking in vitamin D, high in calcium, and low in phosphorus, but adequate in all other respects. Moderate rickets develop in 18 to 21 days. Some of the rats are then fed a known dosage of an International reference standard, and others varying amounts of the food to be tested. In about a week the animals are examined. The amount of food that produces the same amount of healing as does the reference standard is said to contain the same number of units of vitamin D as was contained in the reference standard fed.

The presence of rickets and the progress of healing may be determined in several ways. X-ray pictures of the long bones show lack of calcification of the metaphysis of the bone. As healing proceeds, calcification becomes normal. (See Figs. 40 and 41.) This method is of

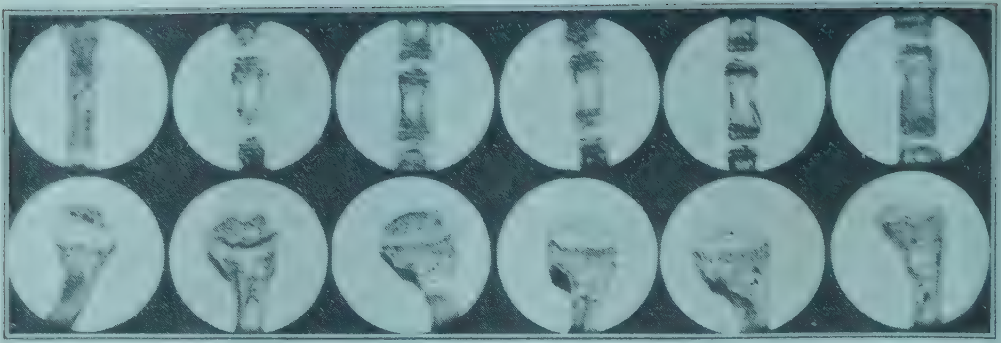


Fig. 40. Healing rickets. Above, tail bones of the rat; below, the femur. Note how calcification proceeds from left to right. Courtesy of Dr. N. B. Guerrant.

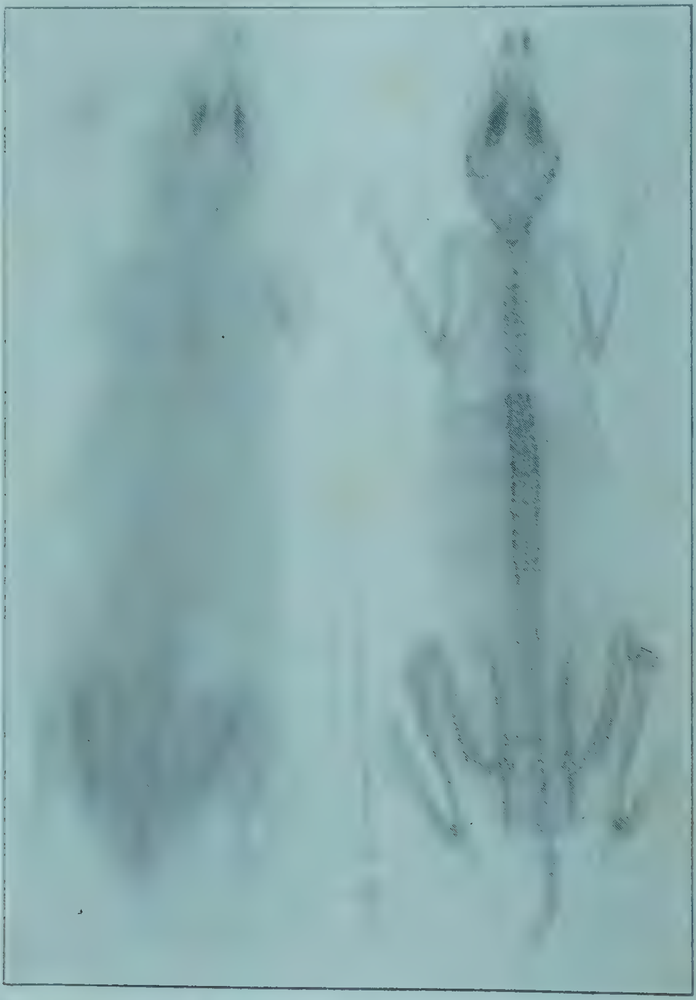


Fig. 41. X-ray pictures of a rachitic and a normal rat, showing that there is poor calcification of all bones in the body in rickets. Courtesy of Dr. N. B. Guerrant.

value because in using it the animal is not injured. A method which is more common is to kill the animal and make a longitudinal section of a leg bone. This section is stained with silver nitrate and exposed to light. The calcified part of the bone is stained. Normal bones have a well-calcified metaphysis and a narrow cartilaginous epiphyseal line. In rickets the metaphysis is poorly calcified and the epiphyseal line is wide and irregular. As healing progresses, the calcification of the metaphysis becomes normal. This test is known as the **line test.**

A third method is to determine ash on fat-free bones. Rachitic bones are much lower in ash than normal bones. By comparing the ash content of a rachitic bone with that of a normal bone for any given age it is possible to estimate the degree of rickets.

Other methods which have been used include the determination of blood-serum phosphorus and the enzyme phosphatase. In rickets phosphorus values are low, and phosphatase values are high. As the rachitic condition improves, these values approach normal. Several colorimetric and physico-chemical methods have been suggested for determining vitamin D but they have not been reliable when applied to various products. The presence of inactive sterols is thought to interfere in these methods.

Units. An International unit of vitamin D is equivalent to 0.025 γ of pure crystalline calciferol.

Cod-liver oil contains about 100 I.U. of vitamin D per gram. Tuna-fish-liver oils contain from 46,000 to 61,000 units per gram. Pure calciferol contains 40,000, and pure vitamin D_3 48,000, units per milligram when rats are used as test animals.

Requirements. There is no definite information as to the vitamin D requirements of a normal adult. For persons who have no opportunity for exposure to clear sunshine and for elderly persons the ingestion of small amounts of vitamin D may be desirable. Other adults probably have little need for vitamin D.

According to the Committee on Food and Nutrition of the National Research Council, the daily allowance for vitamin D should be 400 I.U. for young children. This amount is equivalent to 10 γ of calciferol. The same allowance is suggested for women during the latter half of pregnancy and during lactation. (See Table 12.)

VITAMIN E (α-TOCOPHEROL)

Effects of Deficiency. Vitamin E is a dietary factor which if absent in the foods consumed results in sterility. The early work on this factor was done by Sure and by Evans and Bishop in 1922. They found that certain diets when fed to rats resulted in sterility and that this could be cured or prevented by a proper diet. Lack of vitamin E causes sterility in both male and female rats. In the male sterility is due to a destruction of the germ cells and finally of the seminiferous epithelium so that the sterility becomes permanent. In the female the sterility is temporary, reproduction returning to normal when vitamin E is fed. Sterility in the female is due to a resorption of the fetus. Ovulation, fertilization, and implantation of the ovum on the wall of the uterus occur normally. In poultry, birds which have been fed a diet deficient in vitamin E produce eggs of low hatchability, and those that hatch produce young with low vitality.

Besides interfering with reproduction a lack of vitamin E in the diet may cause degenerative changes in muscles. Muscle fibers atrophy and are replaced by fat and connective tissue. These changes are often accompanied by paralysis. Accompanying these changes is a decrease in muscle creatine and glycogen and an increase in phospholipid and cholesterol. "Stiff lamb" disease is thought to be a result of vitamin E deficiency.

Vitamin E aids in the utilization of vitamin A. When vitamin A deficient rats are fed vitamin A supplements their response is much greater if vitamin E is included in the supplement. It is thought that vitamin E acts as an antioxidant preventing the oxidation of vitamin A in the digestive tract. In this connection it should be mentioned that vitamin E is sometimes added to fats to prevent oxidative rancidity.

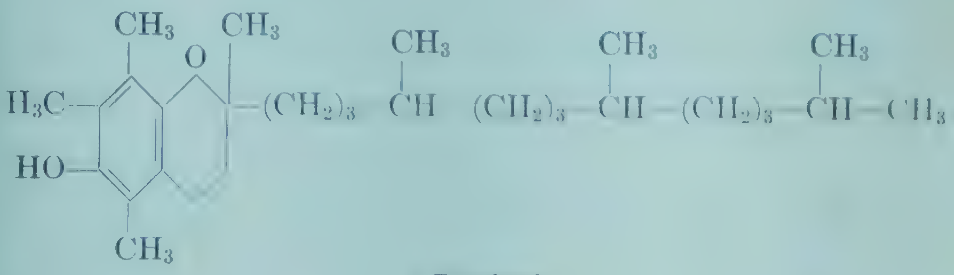
Vitamin E is of little importance in human nutrition because it is so widely distributed in foodstuffs and because it is required in minute quantities. However, it has been used to prevent abortion in women and in cattle.

Sources. Vitamin E is widely distributed in nature. The richest source of this vitamin is wheat-germ oil, where it is concentrated in the nonsterol fraction of the unsaponifiable fraction. Green leaves, cereal grains, and nuts are rich sources of this vitamin. In the animal body it is not present in appreciable amounts in the internal organs, such as the liver, but is found in the muscle and fatty tissues. Milk is not a good source of vitamin E.

Chemical Nature. Vitamin E is the most stable of the vitamins. It is not destroyed by acid or alkali, the process of hydrogenation, or by heating to 180°C. It is partly destroyed by long exposure to ultraviolet irradiation and by oxidizing agents.

It appears that vitamin E is not a single compound. Evans, Emerson, and Emerson have isolated from wheat-germ oil three related compounds which show vitamin E activity. They have been named α-, β-,

γ-tocopherol, of which the α form is the most active. At least one other tocopherol has been reported which shows vitamin E activity. Thus it would appear that vitamin E activity, like vitamin D activity, is due to a certain type of compound, rather than to any one specific compound. Smith, Ungnade, and Prichard have determined the chemical structure of α-tocopherol:



α-Tocopherol

The ring structure in α-tocopherol is called **chromane.** Tied to the ring are four methyl groups, an hydroxyl group, and a phytyl group. β- and γ-Tocopherol differ from α-tocopherol in that they contain three methyl groups tied to the ring instead of four. α-Tocopherol possesses the greatest activity of the three.

Assay. Since sterility is apparently related to other factors besides vitamin E, it has been difficult to develop an adequate method for the assay of this vitamin. However, a biological method which depends upon the curative effect in rats previously fed a diet deficient in vitamin E may be used. Several chemical methods have also been suggested. One measures the intensity of red color produced when tocopherols are oxidized by nitric acid.

An International unit of vitamin E is equivalent to 1 mg. of α-tocopherol acetate. This amount per day, fed for a period of 5 days will prevent resorption of fetuses in 50 per cent of vitamin E deficient rats.

VITAMIN K

Vitamin K was discovered and named by Dam, who called it K because it was associated with blood coagulation. (The German word for coagulation begins with a K.) In this country Almquist was the first to work with this vitamin. Both Dam and Almquist experimented with chickens.

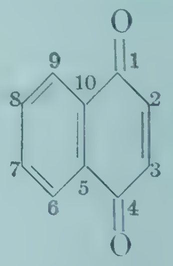
Effects of Deficiency. When vitamin K is deficient in the diet, the time required for the blood to clot is increased. Accompanying slow clotting there is a decrease in the concentration of **prothrombin** in the blood. It will be recalled that prothrombin is a compound associated with blood clotting. It is believed that vitamin K is essential for the formation of prothrombin. The most important symptom of vitamin K deficiency is hemorrhage. In chicks the condition results in bleeding around the pin feathers and hemorrhage into the tissues.

In man there often is a deficiency of vitamin K in obstructive jaundice, where the flow of bile into the intestine is hindered. In the absence of bile vitamin K is not absorbed properly, and thus prothrombin is not synthesized in normal amounts. In gall bladder operations death often results from hemorrhage. Administering vitamin K before this operation greatly reduces mortality rates.

Frequently the prothrombin content of the blood of infants is very low, and death may occur because of hemorrhage resulting from birth injuries. Infant mortality from this cause may be greatly reduced by feeding vitamin K to the infant or to the mother before delivery.

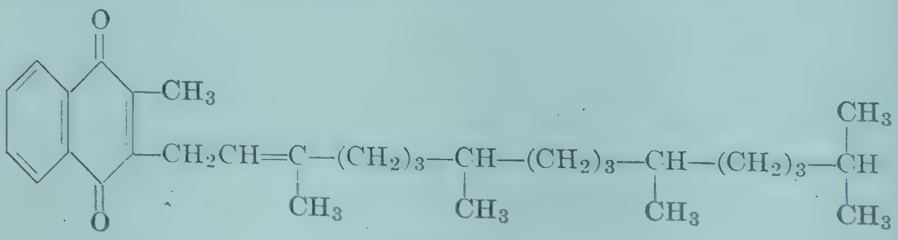
Vitamin K does not prevent all types of hemorrhage. It is effective only where the slow clotting of blood is due to a lack of prothrombin. The hemorrhage of scurvy and of hemophilia is not affected by vitamin K.

Chemical Nature. There are several compounds that show vitamin K activity. Three important ones have been designated **vitamins K_1, K_2, and K_3.** All are derivatives of 1,4-naphthoquinone.



1,4-Naphthoquinone

Vitamin K_1 is 2-methyl-3-phytyl-1,4-naphthoquinone.



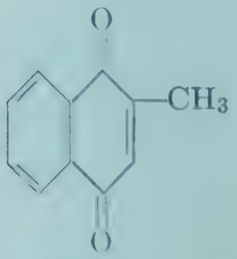
Vitamin K_1

Vitamin K_1 was first isolated from alfalfa-leaf oil.

Vitamin K_2 is found in putrified fish. It differs from vitamin K_1 in that the phytyl side chain is replaced by another side chain.

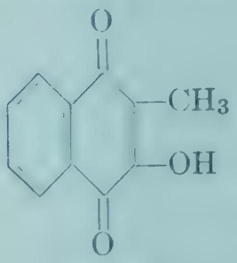
The third variety mentioned, K_3, has been found in corn.

Menadione, which is 2-methyl-1,4-naphthoquinone, is just as potent molecule for molecule as vitamin K_1 itself. Per unit of weight menadione is two and one-half times as potent as vitamin K_1. This is due to the fact that it does not contain the long side chain which is apparently inactive. Thus it appears that the structure present in menadione is the essential structure for vitamin K activity. Menadione is a synthetic product and apparently does not occur in nature. It is used in medicine.



Menadione

Phthiocol, a compound isolated from tubercle bacilli which shows antihemorrhagic properties, is a hydroxy derivative of menadione.



Phthiocol

The vitamins K are fat soluble. They are stable to heat but are destroyed by strong acids and bases and by oxidizing agents. They are sensitive to sunlight and to ordinary electric light.

Sources. The best source of vitamin K is green leaves, such as those of alfalfa, spinach, and oat sprouts. Vegetable oils are also good sources. Cereal grains, carrots, potatoes, and milk, on the other hand, are poor sources of this vitamin. Certain microorganisms found in the intestinal tract are able to synthesize vitamin K_2 and undoubtedly provide an important source of this vitamin in human nutrition.

Assay. In a biological method commonly used, chicks are placed on a vitamin K deficient diet, which is continued until the clotting time of the blood is at least 60 minutes. Then the food to be tested is added to the diet, and its effect on the clotting time is noted. The desired dosage is that which will reduce the clotting time to 10 minutes. By comparing results with those obtained by feeding a known weight of one of the pure vitamin K compounds values may be expressed in terms of that compound. One γ of menadione is equivalent to one unit of the vitamin.

Chemical methods of assay have also been suggested. One depends upon the brownish color produced when the vitamin reacts with sodium ethylate.

Requirements. Since vitamin K is widely distributed in nature, a deficiency of it is rare in normal adults. Little is known about the requirements for this vitamin in health.

Water-Soluble Vitamins

Vitamin B Complex. It has been pointed out that in the early days only two vitamins were thought to exist: one, soluble in fat, called vitamin A; and another, soluble in water, called vitamin B. Vitamin B cured beriberi in man and polyneuritis in other animals. As work progressed, it soon became apparent that the water-soluble fraction of many foods cures or prevents several other diseases. It has now been shown that the water-soluble fraction contains several specific compounds or vitamins, each of which is associated with the cure or prevention of a different disease. Thus what was originally known as vitamin B has become a complex of several vitamins, known as the vitamin B complex. Most of the vitamins present in the vitamin B complex have been isolated in a pure form and their chemical structures determined. It is now customary to refer to them by their chemical names. Another system of naming which has been widely used is to refer to some of the B vitamins by subnumerals, such as B_1, B_2, and B_6. This system of naming is gradually being abandoned.

THIAMINE (VITAMIN B_1)

Effects of Deficiency. One of the first symptoms of thiamine deficiency is a loss of appetite, accompanied by an impairment of the digestive processes. There is a decrease in gastrointestinal motility, which may result in constipation. For this reason the administration of thiamine often alleviates constipation. The loss in appetite is accompanied by loss in weight and fatigue. The loss in weight is due not only to a decrease in food consumption, but also to a lack of the growth-promoting stimulus of thiamine itself. In nursing mothers there is an impairment of growth of the young owing to a deficiency in the milk supply. Sterility may develop in the female because of a cessation of the estrus cycle.

The most striking symptom of thiamine deficiency is the development of a nervous disease known as **polyneuritis**, which, in man, is also called **beriberi.** (See Fig. 42.) Beriberi is characterized by loss of appetite and a progressive loss in weight. The extremities become numb and paralyzed and often swell. This condition is accompanied by definite degenerative changes in the nervous tissues. In animals there is a loss of muscular coordination, a retraction of the head, and finally paralysis. In laboratory work pigeons are used to demonstrate thiamine deficiency rather than rats. In pigeons the polyneuritic symptoms develop early, whereas in rats loss of weight and death may occur before the nervous symptoms develop. If a rat is kept alive by small doses of thiamine, however, the polyneuritic symptoms finally develop. Autopsies of animals dying from thiamine deficiency show important pathological changes in the various organs of the body. One of the most important changes observed is atrophy of the endocrine organs. The adrenal glands, showing hypertrophy, are an exception.

A deficiency of thiamine causes an enlargement of the heart, together with a slowing of the heart beat. Both these conditions are quickly remedied by supplying the vitamin.

Thiamine is closely associated with carbohydrate metabolism. It forms part of an enzyme system which is necessary for the decarboxylation of pyruvic acid, an intermediate compound of carbohydrate metabolism. The enzyme responsible for this decarboxylation is called **carboxylase.** For its action a coenzyme called **cocarboxylase** is necessary. Cocarboxylase is thiamine pyrophosphate. In the absence of thiamine cocarboxylase is not formed and pyruvic acid accumulates in the nervous and other tissues. The neuritis accompanying thiamine deficiency is said to be due to this accumulation of pyruvic acid.

Many cases of neuritis may be due to a thiamine deficiency, and remarkable cures have been reported from administering large doses of this vitamin. Perhaps the best example is the type known as **alcoholic neuritis.** People who drink alcoholic beverages in excess satisfy their appetites with alcohol and do not consume sufficient thiamine-containing foods. If the thiamine deficiency becomes sufficient, they develop a severe neuritis. Spectacular cures may be obtained by injecting large doses of thiamine.

Sources. Thiamine is widely distributed in natural foodstuffs. The best natural sources are brewer's yeast and wheat germ. Eggs and whole grains are good sources of this vitamin. In grains, thiamine is concentrated in the germ and seed coat, and there is relatively little in the endosperm. Hence refined flours are poor sources of this vitamin. Fruits, milk, and vegetables are fairly rich in thiamine. White bread

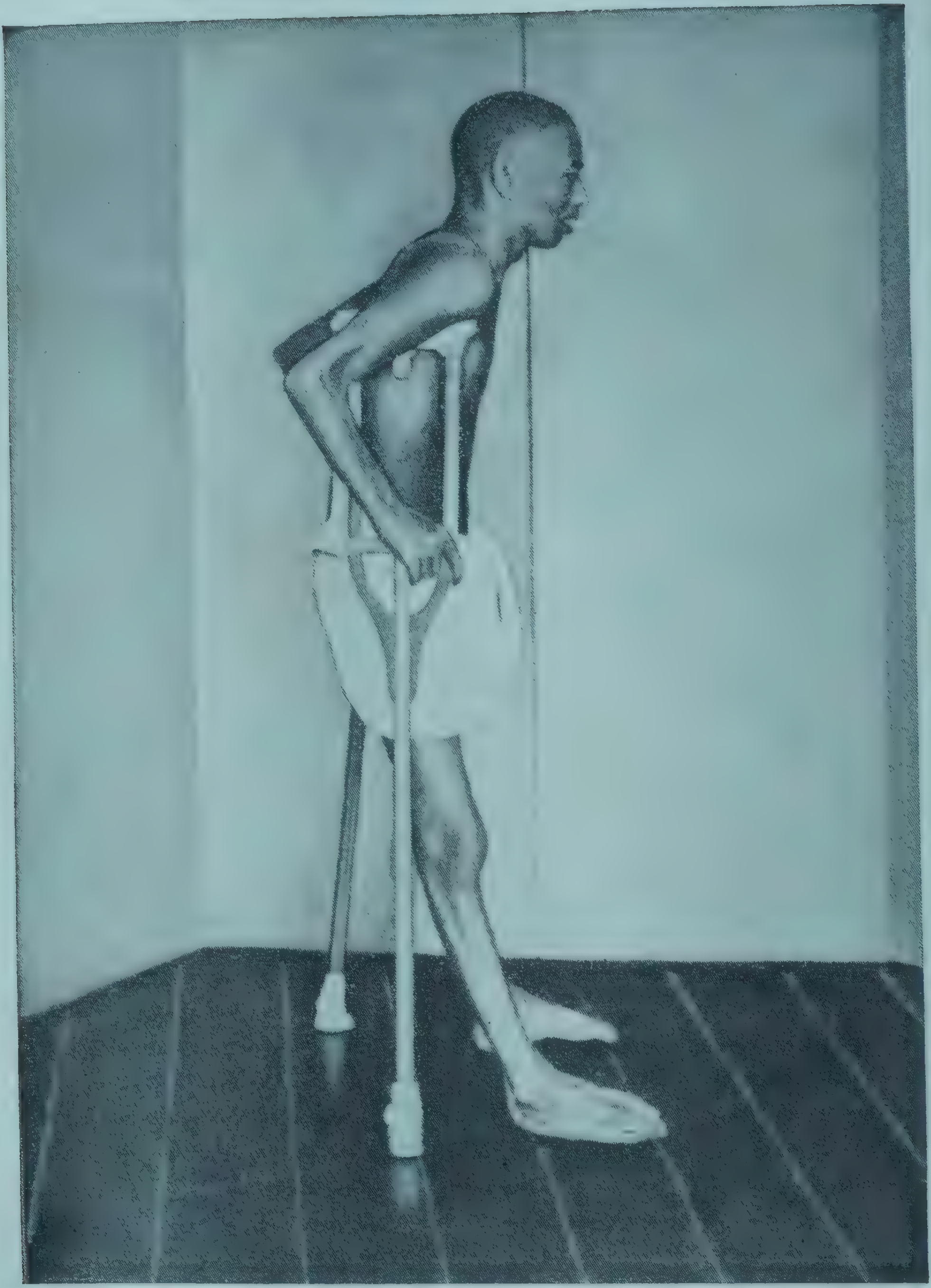


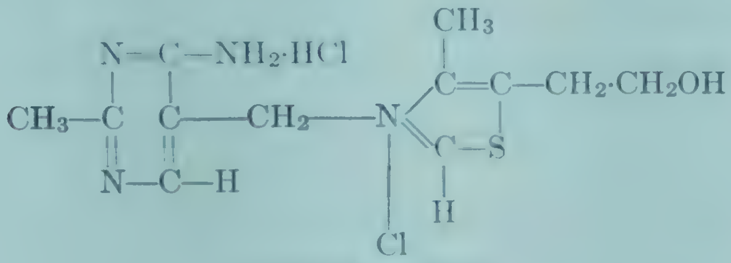
Fig. 42. Atrophic beriberi (Bälz-Miura). From *The Vitamines* by Funk and Dubin. Courtesy of Williams & Wilkins Co.

is a poor source of thiamine, and vegetable fats and oils contain little or none.

Effect of Heat and *p*H on Thiamine. The thiamine content of foods is destroyed to some extent by heat. The hydrogen-ion concentration of the mixture is an important factor in this destruction. The vitamin is much more stable in acid than in alkaline solution. In one experiment, when the food under investigation was heated for 1 hour at 100°C., there was about 10 per cent destruction at a *p*H of 5.2; 30 to 40 per cent destruction at a *p*H of 7.9; 60 to 70 per cent destruction at a *p*H of 9.2; and nearly 100 per cent destruction at *p*H 10.9.

Storage in the Body. Very little thiamine is stored in the body. The absence of this vitamin in the diet causes symptoms of deficiency to appear soon. For this reason it is very important that the food contain a constant supply of this vitamin.

Chemical Nature. The chemical constitution of the antiberiberi factor of the vitamin B complex was determined by Windaus and by Williams. Williams synthesized it and called it **thiamine.** The formula for its hydrochloride is as follows:



Thiamine hydrochloride

The name thiamine was chosen to indicate that the molecule contains sulfur and an amino group. The compound contains two ring structures, the ring to the left in the formula above being pyrimidine and the one to the right thiazole. The two rings are linked together through a methylene group.

Thiamine hydrochloride is a white crystalline compound which melts at 248° to 250°C. It is stable in acid solution and may be heated to 100°C. with very little loss in biological activity. At higher temperatures it gradually decomposes.

Assay. The International unit of thiamine is the activity equivalent to 3γ of the crystalline substance. For the assay of foods various chemical methods have been suggested. The chemical method most generally used is the **thiochrome method.** In this method the thiamine in a food extract is oxidized by potassium ferricyanide in alkaline solution to thiochrome, which gives a bluish fluorescence in ultraviolet light. The intensity of the fluorescence is a measure of the concentration of the thiamine present in the extract.

The most nearly satisfactory methods for measuring the thiamine activity of a food are based upon biological response. One method, which is widely used, is to study the growth response in rats in a manner similar to that described for vitamin A assay. Other methods determine the curative effect on pigeons and rats suffering from polyneuritis. Still another method makes use of the fact that thiamine increases the rate of the heart beat in thiamine deficient rats.

Microbiological methods are also available in which the effect of the vitamin on the growth and metabolism of a microorganism is determined. The organism commonly used is yeast, and the amount of thiamine is estimated from the amount of carbon dioxide produced when a medium containing glucose is fermented under controlled conditions.

Requirements. As was true of vitamin A, there is no definite information concerning the exact optimum requirements of thiamine for man. Rough estimates are available for the thiamine requirements in health, but very little is known about those in disease, although there is evidence to indicate that they may be much greater for a diseased than for a healthy individual. Cowgill believes that the thiamine requirement is proportional to the calorie intake and body weight. Thiamine requirements are greater on a high carbohydrate diet than on a diet high in fat.

According to the Committee on Food and Nutrition of the National Research Council, the recommended daily allowance for thiamine in the diet of a man should be from 1.2 to 1.8 mg., depending upon his activity. (One milligram of thiamine is equivalent to 333 I.U.) For women the allowance is from 1.0 to 1.5 mg. In late pregnancy and during lactation the allowance should be 1.5 mg. Children from birth to 12 years of age should have from 0.4 to 1.2 mg., depending on their age. Girls from 13 to 20 years should have from 1.3 to 1.2 mg., and boys of the same age from 1.5 to 1.7 mg. (See Table 12.)

For a time it was assumed that the average diet contained a sufficiency of thiamine. However, it is now believed that more attention should be paid to the intake of this vitamin. Even on a well-balanced diet the intake of thiamine barely covers the minimum requirements. When we consider that in the manufacture of flour the bran and the germ of wheat are removed and that these are among the richest natural sources of thiamine, it is obvious that the average American is not getting the thiamine that nature intended for man.

RIBOFLAVIN (VITAMIN B_2 OR G)

If yeast, which is a rich source of thiamine, is heated in an autoclave, its thiamine activity is destroyed. However, the resulting material still has biological activity in that it is essential for growth and will cure pellagra in man, blacktongue in dogs, and a pellagralike skin condition in rats, frequently referred to as acrodynia. This heat-stable factor in yeast was originally called vitamin B_2 or vitamin G. We now know that even this is a complex of several vitamins. The name vitamin B_2 was given to this factor by English biochemists to distinguish it from thiamine, which they called vitamin B_1. In the United States the factor was originally called vitamin G.

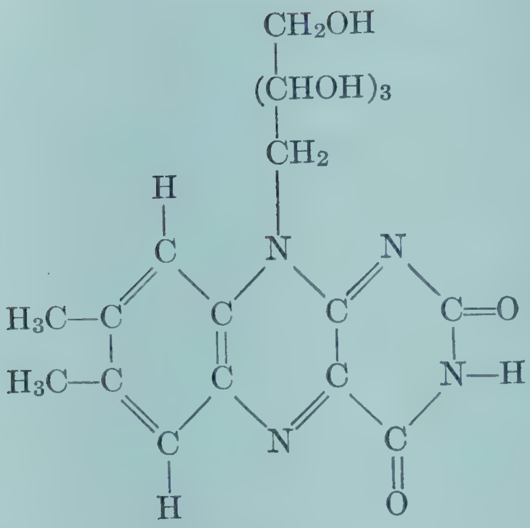
Attempts to isolate pure vitamin B_2 led to the discovery of a crystalline pigment called **riboflavin.** It is a compound consisting of flavin and the pentose sugar D-ribose. Biological tests indicate that riboflavin is the principal growth-promoting constituent of the heat-stable fraction of the vitamin B_2 complex, but that it does not cure pellagra in man, blacktongue in dogs, or the pellagralike skin condition in rats. At the present time riboflavin, vitamin B_2, and vitamin G are considered identical. In the past riboflavin has been called lactoflavin, ovoflavin, and hepatoflavin, depending upon whether it was obtained from milk, eggs, or liver, respectively.

Effects of Deficiency. The main effect of riboflavin deficiency in the diet in young animals is retarded growth. In humans other symptoms are the development of sores and cracks at the corners of the mouth and eye trouble. The eyes become inflamed, and there is dimness of vision and sensitivity to light. In poultry, riboflavin deficiency diminishes egg production and hatchability and causes "curled-toe paralysis," which interferes with walking and eventually causes death. In rats a characteristic dermatitis develops with loss of hair. Cataracts have been observed in rats and swine.

Chemical Structure. Riboflavin was first isolated in 1933 by Kuhn. In 1935 it was synthesized by Kuhn and by Karrer. It is an orange-red solid and is widely distributed in low concentrations in both plants and animals. In dilute solution it appears greenish yellow and shows a greenish yellow fluorescence in ultraviolet light. It is the substance responsible for the greenish color of egg white and milk whey.

Its chemical structure is indicated by the formula on p. 424.

Riboflavin is stable to heat, acids, and oxidizing agents but is destroyed by alkalies and light. It has been estimated that as much as 60 per cent of this vitamin in foods may be destroyed by cooking.



Riboflavin (vitamin B_2 or G)

Physiological Action. Riboflavin functions in the body as a constituent of several enzymes which are associated with biological oxidations and reductions. It is a constituent of an enzyme discovered by Warburg and Christian, their so-called **Yellow enzyme,** which consists of riboflavin, phosphoric acid, and a protein. It is also a constituent of several other oxidative enzyme systems such as D-amino acid oxidase, xanthine oxidase, and succinic acid dehydrogenase.

Riboflavin itself is not active but must be combined with phosphoric acid before it becomes active. This phosphorylation takes place in the intestinal lining, liver, and blood. The phosphoric acid is attached to the terminal carbon atom of the D-ribose portion of the molecule. Riboflavin phosphate functions as a coenzyme and is the prosthetic group of several enzymes. The various enzymes differ with regard to the apoenzymes with which they are associated. The manner in which riboflavin functions as a hydrogen carrier in biological oxidations was discussed on p. 199.

Sources. As would be expected, riboflavin is usually found associated with thiamine. Yeast is perhaps the best source of riboflavin. Other good sources are milk, beef liver, egg yolk, leafy vegetables, and beef muscle. Cereal grains, such as wheat, corn, and rice, contain smaller amounts of this vitamin, which is found to be more concentrated in the germ and bran than in the endosperm. Egg white contains riboflavin but not thiamine.

Assay. The riboflavin content of foods may be determined by biological or chemical methods. In the biological method depleted young rats are fed various amounts of the food to be tested. Other rats are fed a ration containing a known weight of the pure vitamin. The amount of feed that will give the same growth response as the standard contains the same amount of riboflavin as is present in the standard.

A chemical method depends upon the intensity of fluorescence of an extract of the food in ultraviolet light.

A microbiological method measures the amount of lactic acid produced when certain lactic acid bacteria act on a suspension of the food in water. These bacteria require riboflavin and produce lactic acid in amounts proportional to the riboflavin present.

Requirements. The daily allowance of riboflavin recommended by the Committee on Food and Nutrition of the National Research Council is, for adults, 1.5 mg. This quantity should be increased to 2.5 mg. for women during late pregnancy and to 3.0 mg. during lactation. For children up to 12 years the allowance should be from 0.6 to 1.2 mg., depending upon age. Girls from 13 to 20 years should receive from 2.0 to 1.8 mg., and boys of the same age from 2.0 to 2.5 mg. (See Table 12.)

Niacin (Nicotinic Acid, Nicotinic Acid Amide, P–P Factor)

Although nicotinic acid has been known to the organic chemist for nearly a century it was not until recently that its importance in nutrition was appreciated. It was first prepared in 1867 by Huber from nicotine. In 1911 Funk found it in rice polishings but did not realize its value because it did not prevent or cure beriberi. In 1928 Goldberger and co-workers demonstrated that foods rich in "water-soluble B" were effective in alleviating the symptoms of **pellagra** in man and **blacktongue** in dogs. They suggested the name **P–P factor** for the pellagra-preventing constituent of the food. In 1937 Elvehjem and co-workers isolated from liver **nicotinic acid amide,** which they found to be a cure for blacktongue in dogs. It is now known that this compound and nicotinic acid will alleviate many of the symptoms of pellagra in man. Human pellagra and blacktongue in dogs are today considered to be diseases caused by the same dietary deficiency. Both nicotinic acid and its amide are active, the latter being preferred in medical practice because it is better tolerated than the former. In order not to confuse these compounds with nicotine they have been given the names **niacin** and **niacin amide.**

Effects of Deficiency. When the diet of humans is lacking in niacin the disease known as pellagra results. The name comes from an Italian word meaning "rough skin." Pellagra is prevalent among the poorer classes in Italy and in southern United States. The disease starts with inflammation of the skin on the back of the hands, forearms, and neck.

The skin becomes brown and scaly, and finally open sores develop. The tongue becomes red and swollen, and ulcers appear beneath it. Digestive disturbances accompanied by intestinal ulcers, nausea, and diarrhea are common. Nervous symptoms such as insomnia and headache appear, and in advanced cases insanity results.

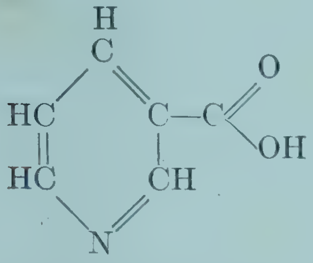
The reason why pellagra is so prevalent in the southern states is that among the poorer classes corn, molasses, and salt pork are the main foods consumed. Dairy products, eggs, vegetables, fruits, and fresh meats are eaten in only limited amounts. Corn is low in niacin, and since it is one of the main foods it is obvious why pellagra is common.

Although niacin appears to be the primary factor involved in pellagra prevention, it has been found that much better results are obtained in treating pellagra if thiamine, riboflavin, and pyridoxine are included in the diet. Thus pellagra may be due to a lack of several factors, not to niacin deficiency alone.

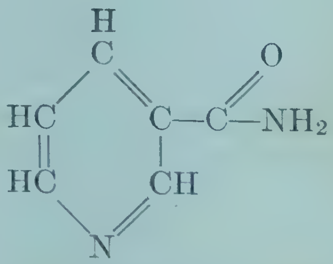
When dogs are fed a diet deficient in niacin they develop a condition known as blacktongue. The symptoms are similar to those found in human pellagra; the tongue becomes swollen, the amount of saliva is increased, diarrhea is produced, and skin lesions appear.

Although rats require niacin they are apparently able to synthesize it provided tryptophan is present in their diet. Rats on a niacin-free diet excrete niacin in their urine, which indicates that it must have been synthesized. On a diet rich in tryptophan a rat requires little niacin in its food.

Chemistry. Niacin is a derivative of pyridine in which a carboxyl group has replaced a hydrogen on the β-carbon atom. Its chemical name is pyridine-β-carboxylic acid or pyridine-3-carboxylic acid. Niacin amide is the amide of niacin.



Niacin (nicotinic acid)

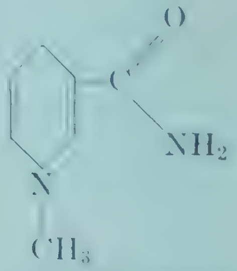


Niacin amide (nicotinic amide)

Niacin is one of the most stable of the vitamins. It is quite stable to acids, alkalies, oxidizing agents, heat, and light.

Physiological Action. The most important function of niacin in the body is that in the form of its amide it constitutes the active group of coenzymes I and II which act as hydrogen carriers in biological oxidations. (See p. 198.) Coenzyme I is a dinucleotide consisting of adenine, niacin amide, and two molecules of D-ribose phosphate; coenzyme II differs from coenzyme I in that it contains three instead of two molecules of phosphoric acid. It would appear, then, that a lack of niacin in the diet interferes with biological oxidations.

When niacin amide is fed to certain species, including man and rats, a portion of it is excreted in the urine as a methyl derivative called N-methyl niacin amide.



N–Methyl niacin amide

In such species large doses of the vitamin produce toxic symptoms. It is thought that the toxic symptoms are caused by the loss of methyl groups from the tissues, which are necessary for transmethylation reactions. When methionine, a good source of methyl groups, is fed with the vitamin the toxic symptoms are suppressed. Possibly large doses of the vitamin are toxic because they deplete the tissue supply of methionine, an essential amino acid.

Sources. One of the best sources of niacin is liver. Other good sources are yeast, lean meats, beans, peanuts, soybeans, and wheat germ. Poor sources are white flour, corn meal, and fats.

Requirements. The daily allowance of niacin recommended by the Committee on Food and Nutrition of the National Research Council is, for men, from 12 to 18 mg., depending on their activity. For women the allowance is from 11 to 15 mg. For children up to 12 years the allowance varies from 4 to 12 mg., depending on age. For girls from 13 to 20 years the allowance should be from 13 to 12 mg., and for boys of the same age from 15 to 17 mg. (See Table 12.)

Assay. The biological method for niacin assay is based upon the curing of blacktongue in dogs. Since rats are able to synthesize niacin in their intestines, through the action of microorganisms, they are not used for assaying this vitamin. Dogs are fed a diet deficient in niacin until blacktongue develops; then the food to be tested for its niacin content is added to the diet, and the amount required to cure the disease is noted. A good chemical method depends upon the fact that niacin reacts with cyanogen bromide and aniline to form a yellow compound which may be determined colorimetrically. Microbiological methods are also available.

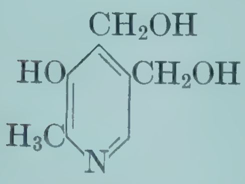
Enriched Flour. During the milling of wheat to produce flour a considerable proportion of the vitamins originally present in the wheat is lost. In order to overcome this objection to the use of white flour many millers are placing on the market a flour to which has been added thiamine, riboflavin, niacin, and iron. To be sold as enriched flour, 2.0 to 2.5 mg. of thiamine, 1.2 to 1.5 mg. of riboflavin, 16 to 20 mg. of niacin, and 13.0 to 16.5 mg. of iron must be added per pound of flour. It should be noted that during milling other factors are removed from wheat which are not compensated for by the above additions. Thus enriched flour does not equal the original wheat nutritionally. At the present time the government is contemplating the enrichment of corn meal and rice for the benefit of those who live largely on these foods.

Pyridoxine (Vitamin B_6)

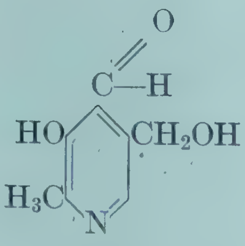
At one time it was thought that the pellagralike skin disease in rats known as **acrodynia** was analogous to pellagra in man. Since niacin amide does not cure the skin condition in rats and since vitamin B_2 complex does, it is evident that still another factor is involved. This factor was first called vitamin B_6; but now, since its chemical nature is known, it is called pyridoxine.

Pyridoxine was isolated in crystalline form by Keresztesy and Stevens in 1938. These investigators and also Kuhn and his co-workers determined its chemical structure in 1939.

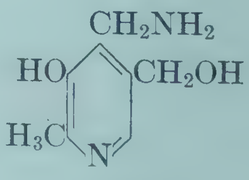
Two other compounds, chemically related to pyridoxine, have biological properties similar to pyridoxine. They are **pyridoxal** and **pyridoxamine.**



Pyridoxine



Pyridoxal



Pyridoxamine

A glance at the formula for pyridoxine shows that it is a pyridine derivative and is thus closely related chemically to the pellagra-preventive factor, niacin. Pyridoxal and pyridoxamine differ from pyridoxine with regard to the group attached to the number 4 position of the pyridine ring; in pyridoxal it is an aldehyde and in pyridoxamine an amine.

Pyridoxine is not affected by heat, acid, or alkali but is destroyed by oxidizing agents.

Effects of Deficiency. Lack of pyridoxine in the diet produces in rats a skin condition in which the nose, tips of the ears, and feet lose their hair and become red and swollen. Other conditions which have been observed in other animals include anemia and fits of an epileptic type.

The role of pyridoxine in humans is not definitely known, but it is said to be effective in treating certain skin conditions. It is possible that in animals in which the requirement for the vitamin is small it is synthesized by bacteria in the intestinal tract.

Physiological Action. Pyridoxine appears to be a part of coenzymes involved in amino acid metabolism. It is the precursor of the coenzyme of four decarboxylases, namely, those acting on arginine, lysine, tryptophan, and tyrosine. Pyridoxal and pyridoxamine are part of coenzymes involved in transamination.

Sources. An excellent source of pyridoxine is brewer's yeast. Other good sources are liver, pork loin, leg of lamb, lean beef, and whole-wheat bread. Milk and leafy vegetables contain appreciable quantities of this vitamin. Pyridoxine usually occurs in foods in combination with proteins although some is present in the free state.

Assay. Pyridoxine may be assayed by determining its curative effect on acrodynia in rats or by determining its growth-promoting properties on certain bacteria. A chemical method depends on the color produced when pyridoxine reacts with a derivative of quinone. One rat unit has been defined as the daily requirement for the prevention or cure of acrodynia, which is equivalent to 10γ of pure pyridoxine.

Requirements. Little is known about the human requirements for pyridoxine. It has been suggested that 1.9 mg. per day is adequate.

Pantothenic Acid

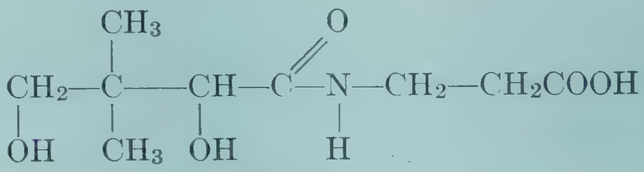
In 1901 Wildier reported that yeast required a specific nutrient factor for growth and called this factor "bios." Subsequent work has shown that "bios" is not a single factor but a complex composed of several factors.

In 1933 R. J. Williams described a substance, widely distributed in nature, which stimulated the growth of yeast cells. He called the substance pantothenic acid from a Greek word meaning "everywhere."

Effects of Deficiency. Pantothenic acid is essential in the diet of chickens; if it is deficient in the diet of hens they produce eggs of low hatchability. If it is deficient in the diet of chicks they develop a dermatitis. Scabs appear at the corners of the mouth, and the neighboring skin becomes inflamed. The chick dies in 2 or 3 weeks. Feeding calcium pantothenate cures the condition.

Pantothenic acid also appears to be essential in the diet of rats, foxes, dogs, and swine. In rats and foxes black hair turns gray when pantothenic acid is eliminated from the diet. The gray hair turns black again when calcium pantothenate is fed. In dogs there are respiratory disturbances, degeneration of the kidneys and liver, and inflammation of the digestive tract. In swine there is a rough dermatitis and ulceration of the digestive tract. It is believed that pantothenic acid is important in human nutrition, but little information is available on this subject.

Chemical Composition. Pantothenic acid has been isolated in pure form, and its chemical structure determined. It may be described as a peptide combination of β-alanine and α,γ-dihydroxy-β-dimethylbutyric acid.



$$\underset{\underset{\mathrm{OH}}{|}}{\mathrm{CH_2}}-\overset{\overset{\mathrm{CH_3}}{|}}{\underset{\underset{\mathrm{CH_3}}{|}}{\mathrm{C}}}-\underset{\underset{\mathrm{OH}}{|}}{\mathrm{CH}}-\overset{\mathrm{O}}{\overset{/\!/}{\mathrm{C}}}-\underset{\underset{\mathrm{H}}{|}}{\mathrm{N}}-\mathrm{CH_2}-\mathrm{CH_2COOH}$$

Pantothenic acid

Since preparations of pantothenic acid are syrupy liquids, it is customary to use the vitamin in the form of the calcium salt, which is a white solid.

Pantothenic acid is fairly stable to heat in neutral solution but is easily hydrolyzed by acid or alkali. It is no longer active after hydrolysis. Being water soluble, much of the pantothenic acid of a food may be lost in cooking if the cooking water is poured off.

Physiological Action. Little is known concerning the mode of action of pantothenic acid. It is thought to be associated with enzymes involved in fat and carbohydrate metabolism and with the process of acetylation. On diets deficient in this vitamin fatty livers develop and there is a decrease in the ability of the liver to oxidize pyruvic acid. Pantothenic acid has been shown to be part of a coenzyme essential for the formation of acetylcholine and for other acetylation reactions. This coenzyme is known as Coenzyme A because of its role in biological acetylations.

Sources. The best sources of pantothenic acid are yeast, liver, and egg yolk. Other good sources are lean meats, whole wheat, green leafy vegetables, sweet potatoes, and cauliflower. Egg white and fruits are poor sources of this vitamin.

About 80 per cent of the pantothenic acid present in foods is in a bound form and is liberated during digestion by enzyme action.

Assay. Biological methods for the assay of pantothenic acid have been developed. One depends upon the cure of the gray-hair condition in black rats which have been fed a diet deficient in the vitamin. Another depends upon growth response in chicks.

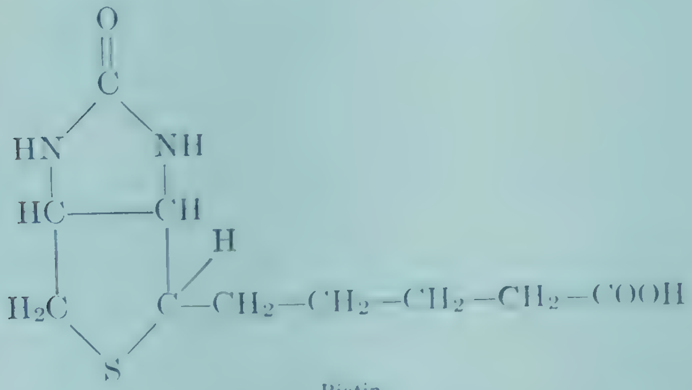
A microbiological method, which gives more quantitative results than the biological method, has been developed. In this method the effect of the vitamin on lactic acid production by certain bacteria is determined.

Requirements. Little is known about the requirements for pantothenic acid of humans. It has been suggested that 10 mg. per day should be sufficient.

Biotin

In the previous section it was pointed out that Wildier's "bios" was a complex composed of several factors. In 1936 Kögl and Tonnis isolated from egg yolk a compound that stimulated the growth of yeast which they called **biotin.** Later biotin was shown to be identical to previously described factors known as vitamin H, coenzyme R, and the anti-egg white injury factor.

Chemical Structure. Our knowledge of the chemical structure of biotin is due largely to the work of du Vigneaud and his co-workers. They have shown it to be a cyclic compound containing two rings, one a cyclic ureid and the other a reduced thiophene ring. A valeric acid side chain is attached to the reduced thiophene ring.



Biotin

Kögl worked with biotin prepared from liver. According to Kögl biotin from egg yolk is different from that from liver, and he suggests that the former be called α-biotin and the latter β-biotin. There is evidence that other compounds may also show biotin activity.

Biotin is a very stable compound in neutral or acid solutions but is easily destroyed by strong alkali or oxidizing agents.

Effects of Deficiency. Besides being necessary for the growth of yeast and other microorganisms biotin is essential in the diet of animals. It is difficult to demonstrate biotin deficiency in animals because the vitamin is synthesized by intestinal microorganisms. However, if certain sulfa drugs are administered the microorganisms are destroyed and biotin synthesis is inhibited. Under these conditions symptoms of biotin deficiency appear if biotin is not present in the food.

One of the first symptoms of biotin deficiency in young animals is cessation of growth. This is followed by a typical dermatitis. Scabs develop around the mouth and between the toes, and the eyelids may become granular and stick together. Rats, chicks, and human beings develop a similar dermatitis when fed large quantities of raw egg white. This so-called **egg white injury** may be prevented by boiling the egg white before feeding it or by feeding biotin. It is believed that raw egg white contains a protein called **avidin,** which combines with biotin and prevents its absorption. When biotin is not absorbed in sufficient quantity, a dermatitis results.

Physiological Action. Biotin appears to be necessary in order for certain biochemical reactions to occur. It may serve as a coenzyme or be necessary for enzyme synthesis. Biotin is necessary for (1) the decarboxylation of oxaloacetic acid to form pyruvic acid, (2) for the formation of oxaloacetic acid from pyruvic acid and carbon dioxide, (3) for the deamination of certain amino acids, and (4) for the synthesis of oleic acid by certain lactic acid producing bacteria. It is thus essential for several important biological reactions.

Sources. Biotin is widely distributed in foods. Excellent sources are egg yolk, yeast, liver, and milk. It is also found in vegetables, cereals, and nuts. For man and some other animals synthesis by bacteria in the intestinal tract is an important source.

Requirements. On an ordinary diet an adequate supply of biotin, which may be synthesized by microorganisms in the digestive tract, is apparently obtained. Very little is known about the requirements of man for this vitamin. We know that it is necessary for the normal growth of certain yeasts, molds, and bacteria.

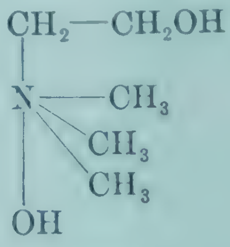
Assay. Foods may be assayed for their biotin content by biological and microbiological methods. In the biological method rats or chicks are fed raw egg white to bring on symptoms of biotin deficiency. Then the food to be tested is fed, and the amount required to cure the disease is noted. By comparing results with those obtained by feeding known quantities of the pure vitamin it is possible to estimate the amount of the vitamin in the food fed.

In the microbiological method the effect of the food on the growth of yeast or on the production of lactic acid by certain bacteria is studied. This method is not widely used.

Choline

Choline has been known for a long time as a constituent of lecithin, but it is only recently that its function as a vitamin has been appreciated. Some persons hesitate to call it a vitamin, since it has been shown that the body may synthesize it, provided methionine or some other compound containing labile methyl groups is available. Choline is a colorless, viscous liquid soluble in water and alcohol. It is usually used in the form of the chloride, which is a white crystalline solid.

Chemical Structure. Choline may be considered a derivative of ammonium hydroxide in which one hydrogen is replaced by the hydroxyethyl group and three hydrogens by methyl groups. Its chemical name is hydroxyethyltrimethylammonium hydroxide.



Choline

Effects of Deficiency. Choline is known to be essential in the diet of rats, chickens, and dogs, and it is possible that other species require it. If lacking in the diet of rats they develop fatty livers. There may also be kidney damage characterized by hemorrhage.

It will be recalled that fats are transported in the body as phospholipids, such as lecithin. Fats are stored in the body as neutral fat molecules. It is obvious that in order to form lecithin, choline must be available. If there is an insufficient supply of choline, fats tend to remain in the storage depots, and the tendency toward the storage of fat is increased.

A disease with which choline is related is **perosis** in chickens and turkeys. In perosis there is a malformation of the bone. The hock joint fails to develop normally, and the leg tendon slips off it, with the result that the bird is unable to walk. Perosis is sometimes called slipped-tendon disease. Both choline and manganese are necessary in the diet to prevent perosis.

Physiological Action. It has just been pointed out that choline is essential for the formation of lecithin, a form in which lipids are transported in the body. This function of choline is often referred to as its **"lipotropic action."**

Another important function of choline is to furnish methyl groups for **transmethylation** reactions. Important compounds in the body such as creatine and methionine which contain methyl groups may be synthesized provided choline and other precursors are present. For example, methionine is an essential amino acid but it can be eliminated from the diet provided choline and homocystine are fed.

A third function of choline is to serve as a precursor for the formation of **acetylcholine,** a compound involved in the transmission of nerve impulses. (See p. 302.)

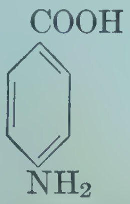
Sources. Choline is found in foods in proportion to their phospholipid content. Egg yolk, liver, kidney, and brain are excellent sources. Meat, peas, beans, leafy vegetables, and the germ of cereals are good sources. Butter, lard, and vegetable oils are poor sources.

Assay. Chemical and microbiological methods are available for assaying foods for their choline content.

Requirements. Little is known concerning the choline requirements for man or animals.

p-Aminobenzoic Acid

Para-aminobenzoic acid, also known as PABA, was first synthesized by Fischer in 1863, but it was not recognized as a vitamin until 1940 when Woods and Fildes showed that it counteracted the bacteriostatic effects of sulfanilamide. (See p. 342.)



p-Aminobenzoic acid

Fildes has shown that it is essential for the growth of many bacteria. Later Ansbacher showed that a lack of this vitamin in the diet of rats produced gray hair. He also showed it to be essential for normal growth of chicks. Sure has reported it to be essential for normal lactation in rats.

The fact that *p*-aminobenzoic acid and pantothenic acid will change gray hair back to its original color in animals has created considerable interest in the use of these vitamins in human nutrition to prevent graying of the hair. At the present time, however, there is no evidence that they have any effect on gray hair in man.

PABA has been used with considerable success in the treatment of Rocky Mountain spotted fever.

Physiological Action. Little is definitely known concerning the physiological action of PABA. It has been suggested that it serves as a coenzyme in certain enzyme systems. It is known to be a constituent of folic acid and may be essential for the synthesis of this vitamin by microorganisms. It is essential for the growth of many microorganisms, and it has been suggested that its role in animal nutrition is to favor the growth of intestinal microorganisms which produce vitamins which in turn are absorbed from the intestinal tract. Thus the action of PABA may be indirect.

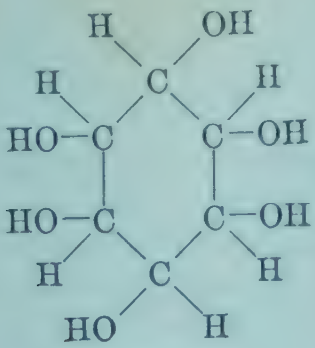
Sources. PABA is rather widely distributed in nature. Yeast, liver, bran, and the germ of cereal and leguminous seeds are good sources of this vitamin.

Assay. Although chemical and biological methods are available for the assay of PABA in foods, microbiological methods are more frequently used.

Requirements. The requirement for PABA in human nutrition is not known.

Inositol

Inositol has been known since 1850, but it was not until 1928 that Eastcott showed it to be essential for the growth of yeast and other microorganisms. In 1940 Woolley found that mice fed a diet containing no inositol failed to grow, lost hair, and became bald in spots. The condition could be cured by feeding inositol. In 1941 Paveck and Baum showed that inositol would cure a baldness around the eyes in rats, known as the "spectacled-eye" condition. The same year Gavin and McHenry showed that a type of fatty liver in rats could be prevented by feeding inositol.



Inositol

Chemistry. Inositol is hexahydroxycyclohexane. Its empirical formula is $C_6H_{12}O_6$, and it is therefore isomeric with simple hexose sugars. Inositol may exist in several isomeric forms some of which are optically active and some of which are not. Only one of these isomers of inositol is biologically active, and it is optically inactive. It is called *meso*-inositol.

Physiological Action. Besides being required for the growth of many microorganisms and preventing baldness in mice and rats, inositol is associated with other important physiological processes. Like choline it is a lipotropic agent, preventing the formation of fatty livers. Inositol is a constituent of certain phospholipids which are thought to aid in lipid transport. It is especially effective in preventing the deposit of cholesterol in the liver.

Inositol has been found to be present in highly purified pancreatic amylase preparations and is thought to be an important constituent of this enzyme system.

It has been demonstrated by means of isotopes that inositol may be converted into glucose in rats. In certain fish inositol is deposited in the liver in place of glycogen. Thus the metabolism of inositol and glucose appear to be interrelated.

Sources. Good sources of inositol are wheat bran, whole grain cereals, and yeast. In wheat bran it occurs as phytin, the calcium-magnesium salt of the hexaphosphate of inositol.

Assay. Inositol is assayed by microbiological methods using yeast as the test organism.

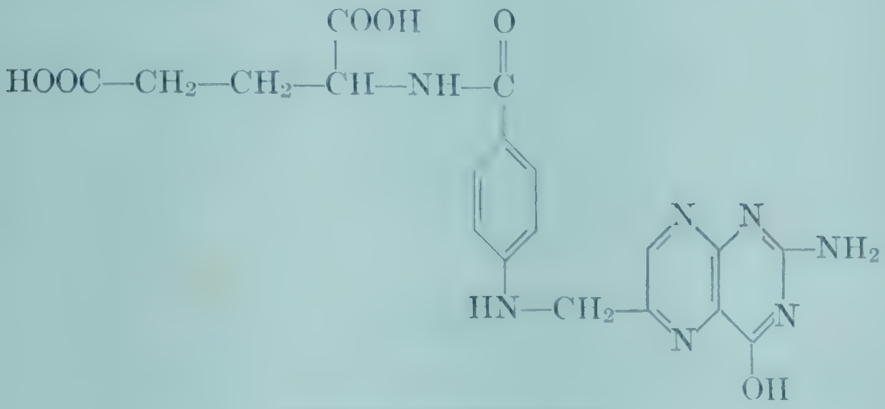
Requirements. The human requirement for inositol is not known.

Folic Acid

The discovery of folic acid, also known as **pteroylglutamic acid** or PGA, is the result of work done by many investigators. It has been known by many names, and only recently has it been appreciated that these various terms refer to the same factor.

In 1932 Wills and Balimoria, working with monkeys, showed that certain types of anemia could be cured by feeding yeast. Later Day and co-workers called this factor "vitamin M." In 1938 Stokstad and Manning reported a factor necessary for the growth of chicks which they called "factor U." In 1939 Hogan and Parrott discovered a factor necessary for normal blood development in chicks and called it "vitamin B_c." In 1940 Snell and Peterson found a factor that stimulated the growth of *Lactobacillus casei* which they called the "*L. casei* factor." A year later Mitchell and co-workers obtained from extracts of green leaves a factor that stimulated the growth of *Streptococcus lactis* which they called "folic acid." It now appears that all of these factors are identical or closely related.

Chemistry. In 1945 Angier and co-workers announced the synthesis of the *L. casei* factor from liver, and they found it to be pteroylglutamic acid.



Folic acid (pteroylglutamic acid)

Pteroylglutamic acid is composed of three simpler molecules. The nitrogen-containing ring structure to the right in the formula is **pteridine.** Attached to this through an amino group is ***p*-aminobenzoic acid.** At the left **glutamic acid** is attached through a peptide linkage. Two other forms of folic acid are known, one containing three and the other seven molecules of glutamic acid in polypeptide chains. The former is called **pteroyltriglutamic acid** and the latter **pteroylheptaglutamic acid or B_c conjugate.** An enzyme capable of hydrolyzing B_c conjugate to pteroylglutamic acid and glutamic acid is called **B_c conjugase.**

Physiological Action. Besides being essential for the growth of certain microorganisms, folic acid is required by chickens, turkeys, guinea pigs, rats, monkeys, and man. In animals it prevents and cures a certain type of anemia. In man it is useful in the treatment of the anemias of pellagra, sprue, and pregnancy. In pernicious anemia it stimulates red cell production but has no effect on the neurological symptoms of the disease. Thus it is not the antipernicious anemia factor. If lacking in the diet of poultry the eggs hatch poorly; in rats lactation is impaired.

Of the three forms of folic acid, pteroylglutamic acid and pteroyltriglutamic acid are equally effective biologically when assayed by microbiological methods. Pteroylheptaglutamic acid is ineffective in stimulating the growth of certain microorganisms. Apparently the enzyme B_c conjugase must be present to hydrolyze it to pteroylglutamic acid before it becomes active. This enzyme is absent in certain microorganisms but present in the tissues of animals which can utilize B_c conjugate.

With regard to the mechanism by which folic acid functions little is known. There is some evidence that it is involved in the synthesis of thymine, a pyrimidine found in nucleic acid. Nucleic acid is necessary for the production of red blood cells. The feeding of thymine has been shown to lower the requirements for folic acid in the diet.

Sources. Folic acid is widely distributed in foods. Green leafy vegetables, grains, yeast, liver, kidney, and beef are good sources.

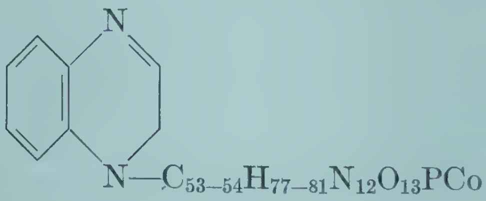
Requirements. Little is known about the requirements of folic acid for man. When taken in large doses it is toxic.

Assay. Biological and microbiological methods are used to assay foods for their folic acid content.

Vitamin B_{12}

In 1948 workers at Merck and Company in the United States and Smith in England isolated a deep red, crystalline compound from liver which is effective in the treatment of pernicious anemia. In the previous section it was pointed out that folic acid stimulates red cell production in pernicious anemia but has no effect on the nervous symptoms. Vitamin B_{12} stimulates red cell production and also controls the nervous symptoms of the disease. It is now considered to be the **anti-pernicious anemia factor.**

Chemistry. Although the complete structure of vitamin B_{12} is not known, some facts are available concerning its composition. It contains cobalt and a cyclic structure, 5,6-dimethylbenzimidazole.



Vitamin B_{12}

Vitamin B_{12} exists in several forms known as vitamins B_{12a}, B_{12b}, B_{12c}, etc. All of these forms are biologically active and appear to differ with regard to the nature of some radical loosely attached to the cobalt atom.

Sources. Vitamin B_{12} is unique in that it is not found in higher plants. It is however synthesized by many microorganisms. It is thought to originate by microbiological synthesis in the digestive tract. From here it is absorbed and deposited in the body tissues, especially those of the liver. Liver and other animal proteins are good sources of this vitamin.

Physiological Action. Little is known as to how vitamin B_{12} functions in alleviating the symptoms of pernicious anemia. One theory is that it stimulates red cell production when in the presence of thymidine, a nucleoside containing thymine. Since folic acid is necessary for the synthesis of thymine the two vitamins are closely related in their action.

Vitamin B_{12} is considered to be the **extrinsic factor** in pernicious anemia. Even though it is produced by intestinal organisms, in pernicious anemia patients it is of no value because of the absence of an intrinsic factor present in normal gastric juice which is thought to be necessary for its absorption. The feces of pernicious anemia patients contain vitamin B_{12}.

Vitamin B_{12} is a very potent substance; as little as 3γ per day will control the symptoms of pernicious anemia in humans.

Vitamin B_{12} stimulates the growth of poultry and pigs and is added to their feeds for this purpose.

Vitamin B_{12} is associated with transmethylation reactions.

Animal Protein Factor (APF). It has been known for some time that vegetable proteins are inferior to animal proteins as feed for pigs and poultry. The assumption has been that the difference was due to variations in the amino acid content of the proteins. It is now believed that it is owing to the fact that there is some factor present in animal proteins that is absent in vegetable proteins. This factor has been called the animal protein factor. As work has progressed it has become apparent that this factor is identical or closely related to vitamin B_{12}.

The animal protein factor is absent in plant foods and yeast but is present in tankage, fish meal, and liver. It is also present in feces, which accounts for the fact that many animals eat their own feces (coprophagy).

Other factors have been described which are said to be present in the vitamin B complex, but so little is known about them that they will not be discussed here.

Ascorbic Acid (Vitamin C)

Effects of Deficiency. Ascorbic acid is a dietary factor which must be present in food in order to prevent the disease known as **scurvy.** It is therefore called the **antiscorbutic vitamin.** The first important work on this vitamin was done by Holst and Frölich in Norway. At one time scurvy was very prevalent in northern Europe, and sailors on a long voyage were commonly afflicted with the disease. It is now known that a lack of fresh vegetables and fruits in the diet is the causal factor.

In human beings the onset of the disease is slow. During the early stages the patient becomes lazy, develops anemia, and loses weight. Later the skin turns brown, the gums become spongy and bleed, and the teeth loosen. The bones become brittle and are easily broken. There may be a marked swelling of the extremities. Hemorrhage is a very important characteristic of the disease. On slight injury hemorrhages develop under the skin. Death from scurvy is often due to internal hemorrhages.

Not all animals seem to require ascorbic acid in their diet. Rats, birds, and cows do not develop symptoms of scurvy when ascorbic acid is removed from their food, and it is possible that they are capable of synthesizing this vitamin. Man, monkeys, and guinea pigs require ascorbic acid. For the study of this vitamin guinea pigs are used.

Physiological Action. Ascorbic acid is necessary for the formation and maintenance of the substance found between the cells which helps to hold them together, especially in the capillary walls, cartilage, bones, and teeth. The loss of intercellular substance on a diet lacking in ascorbic acid is said to be the cause of the structural weaknesses which develop in scurvy. Hemorrhage is due to weakness of the capillary walls, fragile bones to weakness in the bony tissues, and loose teeth to degeneration of the tissue of the jaw bone. The loss of intercellular substance in the teeth may result in tooth decay, and thus a lack of ascorbic acid in the diet may be a contributing factor in dental caries. A large intake of this vitamin in such form as orange juice is believed to be a good preventive of dental caries.

Ascorbic acid is thought to be associated with biological oxidations and reductions taking place in tissues. It may prove to be a coenzyme in some oxidative enzyme system. It is known that ascorbic acid is necessary for the normal metabolism of tyrosine. When the intake of ascorbic acid is low, intermediate products of tyrosine metabolism may appear in the urine.

Ascorbic acid is said to aid the body in resistance to certain diseases. When the intake of this vitamin is liberal, guinea pigs have been found more resistant to tuberculosis and diphtheria toxin than when the intake is low.

Sources. Ascorbic acid is found quite widely distributed in plants. Meats and eggs contain very little. The richest sources are peppers, the citrus fruits, such as oranges, lemons, limes, and grapefruit, tomatoes, and raw cabbage. Rose hips are an exceptionally rich source of ascorbic acid. Although potatoes do not contain the concentration of ascorbic acid found in the foods just mentioned, still they are an important source of this vitamin by reason of the large quantities of potatoes included in the ordinary diet.

The cortex of the adrenal glands and the corpus luteum contain the highest concentration of ascorbic acid of any tissues in the body.

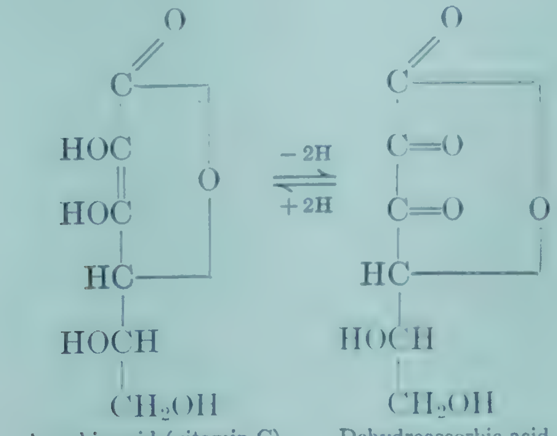
Ascorbic acid is lacking in dry, mature seeds; hence cereals contain very little, if any, of this vitamin. As seeds germinate, there is a marked generation of ascorbic acid. Sprouted grains are therefore a very good source of this vitamin.

Destruction by Heat. The destruction of ascorbic acid by heat is probably an oxidation process. Dutcher and his co-workers found that, when milk is heated to 145° F. for 30 minutes in closed bottles without access to air, there is little destruction of ascorbic acid; but, if it is heated in an open vessel, under the same conditions of time and temperature, with air bubbled through it, destruction is complete. It is evident that pasteurization of milk in an open vat, as sometimes practiced, leads to considerable destruction of ascorbic acid.

Traces of certain metals, especially copper, greatly accelerate the destruction of ascorbic acid; hence copper equipment should not be used in the pasteurization of milk.

Chemical Structure. Our knowledge of the chemical structure of vitamin C is the result of the work of several investigators, notably Szent-Györgyi and Waugh and King, who independently isolated the pure compound, and Reichstein and Haworth, who independently established its chemical structure. It is an oxidation product of a hexose sugar and has been given the name of **L-ascorbic acid.** It is also called cevitamic acid. It has been synthesized from L-xylose and from D-glucose. D-Ascorbic acid is physiologically inactive.

Ascorbic acid readily loses two of its hydrogens to form a ketone called dehydroascorbic acid.



L-Ascorbic acid (vitamin C) Dehydroascorbic acid

From the above formulas it is evident how these two compounds may function as oxidation-reduction catalysts.

Assay. In the biological method for the assay of foods for their ascorbic acid content guinea pigs are used instead of rats. For this determination from 6- to 8-week-old guinea pigs weighing from 250 to 300 grams are fed a basal diet complete in all respects except that it contains no ascorbic acid. Along with this diet are fed varying amounts of the food being tested. That amount of the food which will just prevent scurvy is said to contain the minimum protective dose. This dose is equivalent to approximately 0.5 mg. of L-ascorbic acid or from 10 to 12 International units. An International unit of ascorbic acid is equivalent to 0.05 mg. of L-ascorbic acid.

Besides the biological methods chemical methods are available for determining ascorbic acid. One involves the titration of the vitamin with standard iodine solution; another its titration with a standard solution of a dye, 2,6-dichlorobenzenoneindophenol. The latter titration is said to be the more specific for ascorbic acid.

Requirements. According to the Committee on Food and Nutrition of the National Research Council, the daily allowance of ascorbic acid in the diet of a man should be 75 mg. For a woman it should be 70 mg., and during the latter half of pregnancy this amount should be increased to 100 mg. During lactation a woman should receive 150 mg. Children up to 12 years should receive from 30 to 75 mg., depending on their age. (See Table 12.)

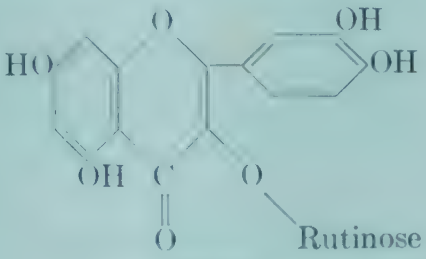
It is important that a nursing mother have in her diet a generous supply of foods containing ascorbic acid in order to supply this factor to her child. The addition of orange juice to the diet of a bottle-fed infant is to compensate for the relatively low supply of this vitamin in cow's milk, which contains only about one-fourth as much ascorbic acid as mother's milk. Orange juice is especially important if the milk is boiled. Brief boiling of milk in an open dish will reduce the ascorbic acid content one-fifth to one-half. Pasteurized milk has a lower ascorbic acid content than raw milk.

Methods are available for determining whether a person is deficient in ascorbic acid. One method tests the resistance of the capillaries to rupture by applying suction to a small area of the skin. If the individual being tested is deficient in ascorbic acid, slight hemorrhages will occur under the skin. Another method determines the ascorbic acid excreted in the urine by a titration method when a test dose of the vitamin is given. If the body is deficient in ascorbic acid, the test dose will be retained by the tissues. If the body supply is already adequate, much of the test dose will appear in the urine. Still another method determines the concentration of ascorbic acid in the blood. A fasting individual should normally have from 1.5 to 2.0 mg. of ascorbic acid per 100 ml. of blood.

Vitamin P (Citrin)

According to Szent-Györgyi, a factor other than ascorbic acid is associated with the hemorrhages found in scurvy. He called this factor vitamin P, because it controls vascular permeability. It has been isolated from red peppers and lemon juice. Chemically vitamin P has been shown to be a mixture of two flavone glycosides, **hesperidin** and **eriodictin,** of which the former is the most abundant. The vitamin has been given the name **citrin.**

A compound similar to citrin in structure and biological properties, called **rutin,** has been isolated from tobacco leaves and buckwheat. It has the following structure:



Rutin

Rutin is a flavone glycoside in which rutinose, a disaccharide composed of glucose and rhamnose, is the sugar.

Vitamin P activity is thus not due to a single compound but rather to a group of compounds similar in structure to rutin.

Vitamin P is found in most foods that contain ascorbic acid so that any diet which is adequate with regard to ascorbic acid should be adequate with regard to vitamin P also. Citrus fruits, green peppers, green leaves, and grapes are good sources.

Antivitamins

An important development in the field of vitamin research has been the study of substances called antivitamins which inhibit the action of vitamins. It will be recalled, in discussing the mechanism responsible for the effectiveness of the sulfa drugs, that sulfanilamide was thought to replace *p*-aminobenzoic acid in an enzyme system essential for the growth of many bacteria. It can do this because of its similarity in structure to *p*-aminobenzoic acid. When this replacement has occurred, the enzyme is no longer active. In other words, *p*-aminobenzoic acid cannot function as it should. Sulfanilamide is thus an antivitamin for *p*-aminobenzoic acid.

An antivitamin might be defined as a compound, similar in chemical structure to a vitamin, which may physically replace the vitamin in some biological system, rendering the system biologically inactive.

Antivitamins are known for thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, folic acid, ascorbic acid, and vitamin K. It is thought that they all act in a similar manner, that is, by blocking the formation of some enzyme or coenzyme.

Antivitamins should prove of value in vitamin research as they may be used to produce vitamin deficiencies in animals without feeding them vitamin-free diets.

Antivitamins should have important medical applications. It is possible that the answer to the cancer problem may lie in this field. In cancer there is a stimulation of cell development which is likely caused by the overactivity of some enzyme. If this enzyme requires a vitamin as a coenzyme for its activity, it would appear that an antivitamin might be found that could block the action of the enzyme. Without the active enzyme cells would not be stimulated and cancer would not develop.

REVIEW QUESTIONS

1. What is a vitamin?
2. Give the early history of vitamins.
3. What is the difference between a vitamin and a hormone?
4. Into what two main classes may vitamins be divided?
5. What are the symptoms of vitamin A deficiency?
6. What is night blindness? What is its cause?
7. Name the important sources of vitamin A.
8. What is the chemical structure of vitamin A?
9. How do α-, ß-, and γ-carotene and cryptoxanthine differ in chemical structure? What structure in these molecules is responsible for vitamin A activity? How do the above compounds differ in their ability to act as precursors of vitamin A in the body?
10. Where are vitamins A_1 and A_2 found?
11. How are foods assayed for their vitamin A content?
12. What is an International unit of vitamin A? What should be the daily allowance of vitamin A in the diet?
13. What are the effects of vitamin D deficiency?
14. Name three types of rickets.
15. Name the factors which are associated with rickets.
16. What is the difference between ergosterol and vitamin D_2?
17. What is vitamin D_3? How does it differ in chemical structure from vitamin D_2?

18. What is viosterol?
19. Discuss the irradiation of foods.
20. What are the important sources of vitamin D?
21. Is there danger in taking large quantities of vitamin D?
22. Is vitamin D stable?
23. How are vitamin D preparations assayed?
24. What is an International unit of vitamin D? What are the vitamin D requirements?
25. What are the effects of vitamin E deficiency?
26. What are the important sources of vitamin E?
27. What is the chemical nature of vitamin E?
28. Is vitamin E important in human nutrition?
29. How may foods be assayed for their vitamin E content?
30. What are the effects of a vitamin K deficiency?
31. Are all types of hemorrhage controlled by vitamin K?
32. Name several compounds which show vitamin K activity and indicate their chemical structures.
33. Name several sources of vitamin K in the diet.
34. How may a food be assayed for its vitamin K activity?
35. Is vitamin K an important consideration in the diet of a normal adult?
36. What vitamins are included in the vitamin B complex?
37. What happens when there is a deficiency of thiamine in the diet?
38. How is thiamine related to carbohydrate metabolism?
39. Name the best sources of thiamine.
40. What is the effect of heat and pH on thiamine?
41. Does the body store thiamine?
42. What is the formula for thiamine?
43. What is an International unit of thiamine?
44. How are foods assayed for their thiamine content?
45. What is the recommended daily allowance of thiamine in the diet?
46. What other names are used for riboflavin?
47. What are the effects of a deficiency of riboflavin in the diet?
48. What is the physiological action of riboflavin?
49. What is the formula for riboflavin?
50. Name the important sources of riboflavin.
51. How may food be assayed for its riboflavin content?
52. What is the recommended daily allowance for riboflavin in the diet?
53. What are the effects of a niacin deficiency in the diet?
54. By what other names is niacin known?
55. What are the chemical formulas for nicotinic acid and nicotinic acid amide?
56. Discuss the physiological action of niacin.
57. Name several sources of niacin.
58. What is the recommended daily allowance for niacin in the diet?
59. How may a food be assayed for its niacin content?
60. What is meant by enriched flour?
61. Write the formulas for pyridoxine, pyridoxal, and pyridoxamine.
62. What are the effects of a deficiency of pyridoxine in the diet?
63. What are the physiological actions of pyridoxine, pyridoxal, and pyridoxamine?
64. Name several sources of pyridoxine.

65. How may a food be assayed for its pyridoxine content?

66. Discuss pantothenic acid as to physiological function, chemical structure, sources, methods of assay, and daily allowances.

67. What are the effects of a biotin deficiency in the diet? What is the cause of "egg white injury"?

68. What is the chemical structure of biotin?

69. Name several physiological activities of biotin.

70. Name several foods that are good sources of biotin.

71. How may foods be assayed for their biotin content?

72. What is the formula for choline?

73. What are the effects of a deficiency of choline in the diet?

74. Discuss the physiological action of choline.

75. Name several good food sources of choline.

76. What is the formula for *p*-aminobenzoic acid?

77. What happens when there is a deficiency of this vitamin in the diet o rats?

78. Can gray hair in humans be prevented by vitamins?

79. What is the relationship between *p*-aminobenzoic acid and sulfa drugs?

80. Name several foods which are good sources of PABA.

81. What is the formula for inositol?

82. What are the effects of an inositol deficiency in the diet of mice and rats

83. Discuss the physiological action of inositol.

84. Name several foods which are good sources of inositol. What is phytin?

85. What is the chemical structure of folic acid?

86. What are B_c conjugate and B_c conjugase?

87. In what types of anemia is folic acid effective as a treatment?

88. Is folic acid the antipernicious anemia factor?

89. Name several foods which are good sources of folic acid.

90. What mineral element is present in vitamin B_{12}?

91. Is vitamin B_{12} found in higher plants? Name several good sources o vitamin B_{12}. How does vitamin B_{12} originate?

92. Discuss the role played by vitamin B_{12} in the treatment of perniciou anemia.

93. Discuss the animal protein factor.

94. What are the effects of an ascorbic acid deficiency in the diet?

95. What is the physiological action of ascorbic acid?

96. What are the important sources of ascorbic acid?

97. What is the effect of heat on ascorbic acid?

98. What is the chemical nature of ascorbic acid?

99. How may foods be assayed for their ascorbic acid content?

100. What should the daily allowance for ascorbic acid in the diet be?

101. Where is vitamin P found, and with what pathological condition is i associated?

102. What is rutin and what is its chemical structure?

103. What are antivitamins? How do they function?

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